

### U.S. EPA REGION IV

# SDMS

### POOR LEGIBILITY

PORTIONS OF THIS DOCUMENT MAY BE DIFFICULT TO VIEW DUE TO THE QUALITY OF THE ORIGINAL.

## TO MAKE THE DOCUMENT READABLE, TRY ONE OR MORE OF THE FOLLOWING:

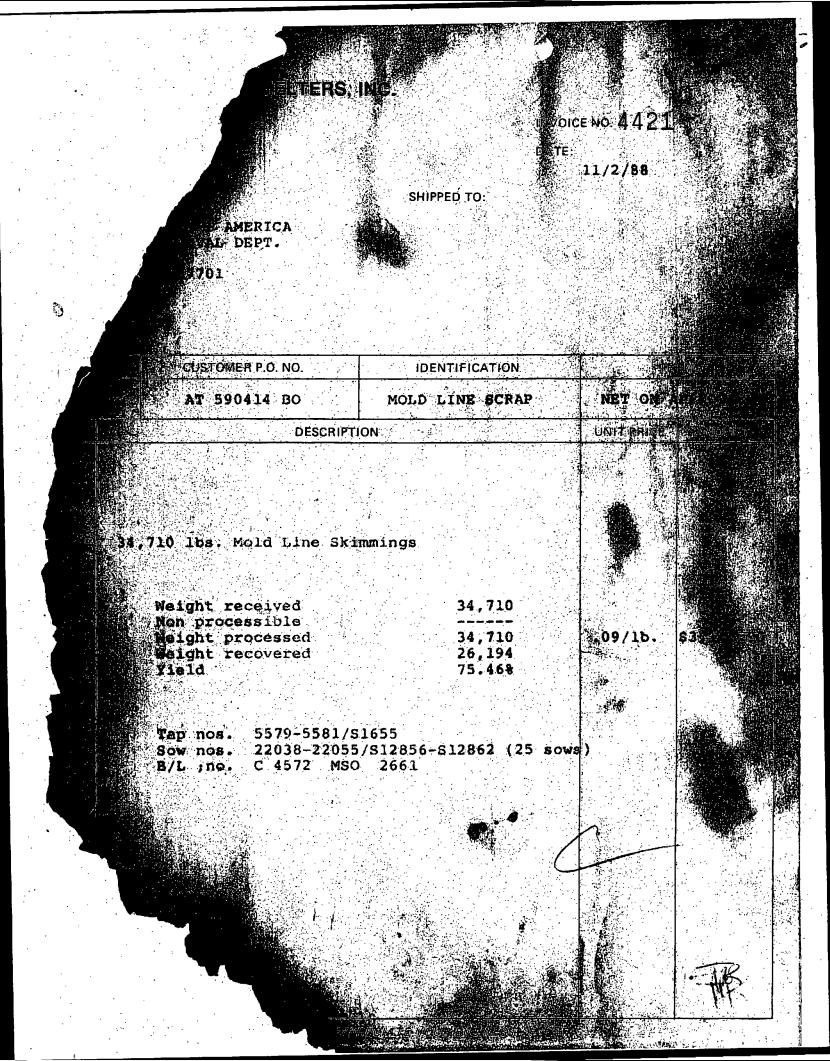
#### From the Displays Settings in Windows Control Panel:

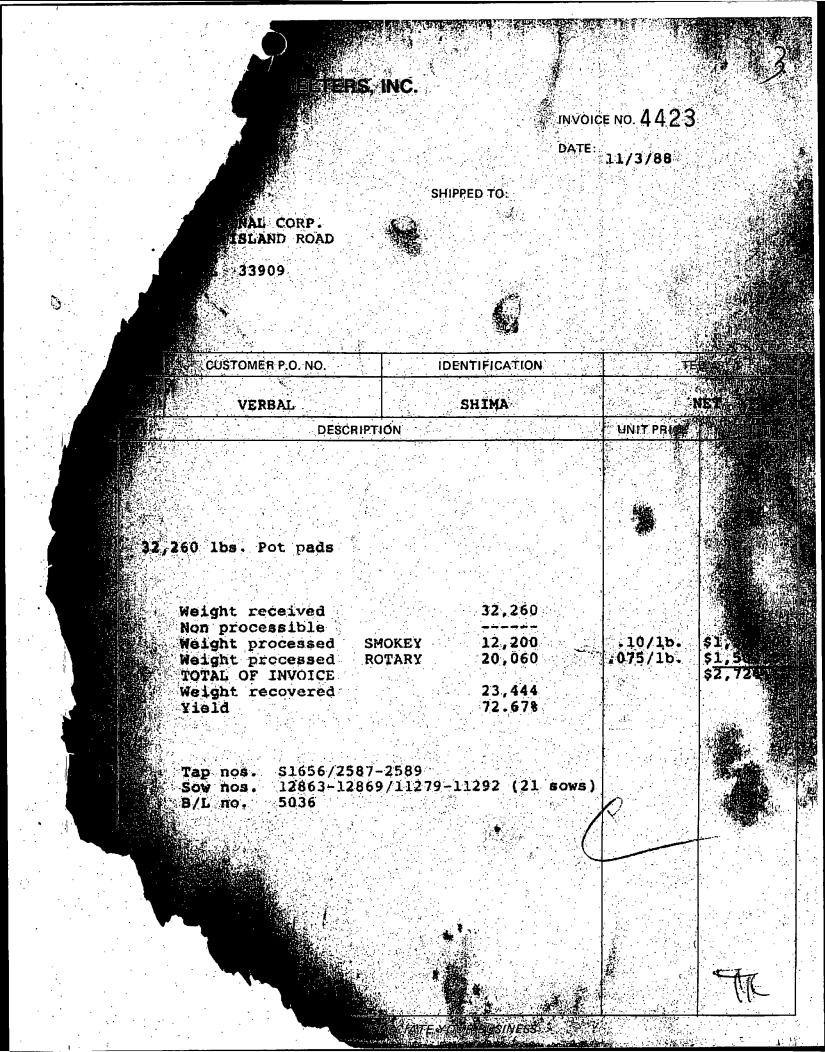
- 1. Set the Color Quality to the highest available: 24 bit or 36 bit.
- 2. Increase or decrease the Screen resolution.

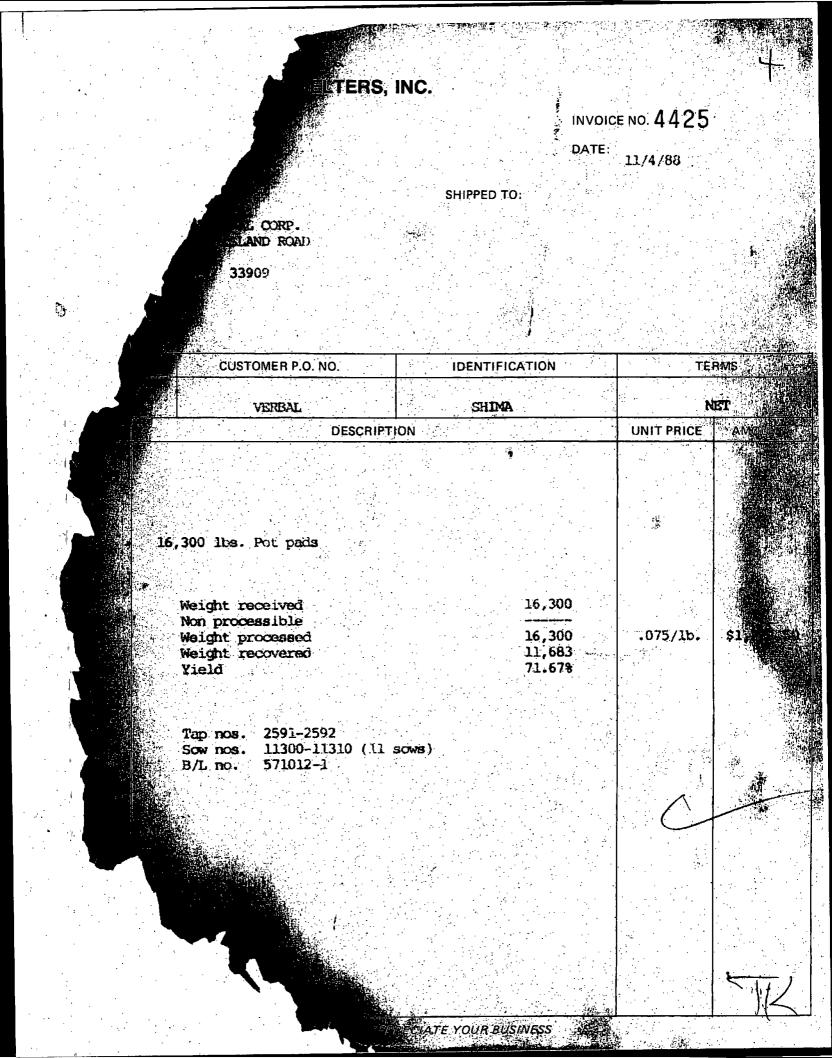
#### From the Monitor/Display Controls:

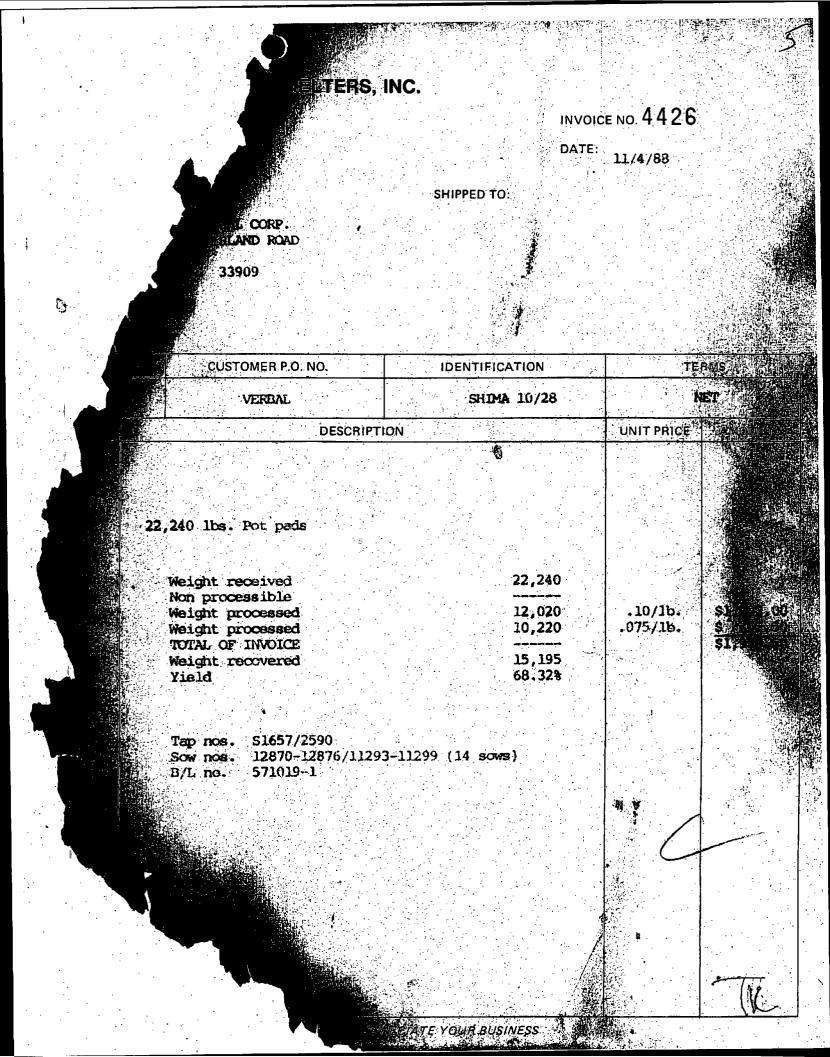
- For dark image page, increase the brightness and decrease the contrast.
- 2. For light image page, decrease the brightness and increase the contrast.

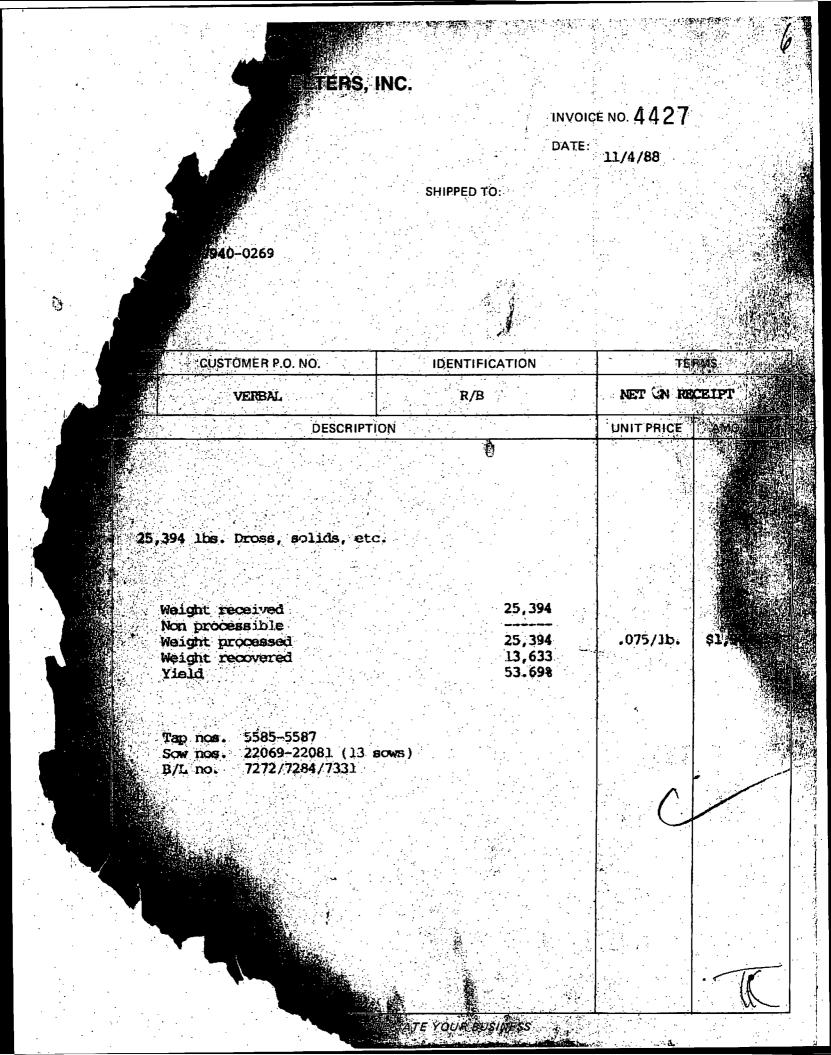
\*\* PLEASE CONTACT THE APPROPRIATE RECORDS CENTER TO VIEW THE MATERIAL\*\*

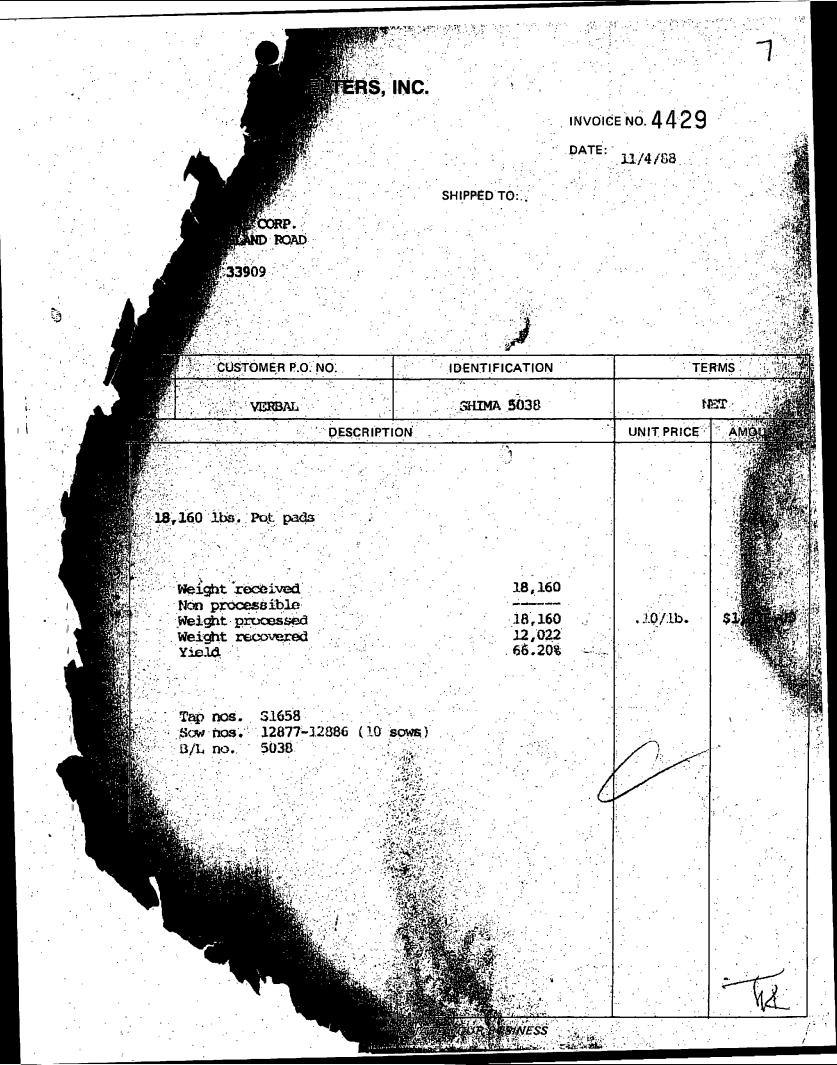


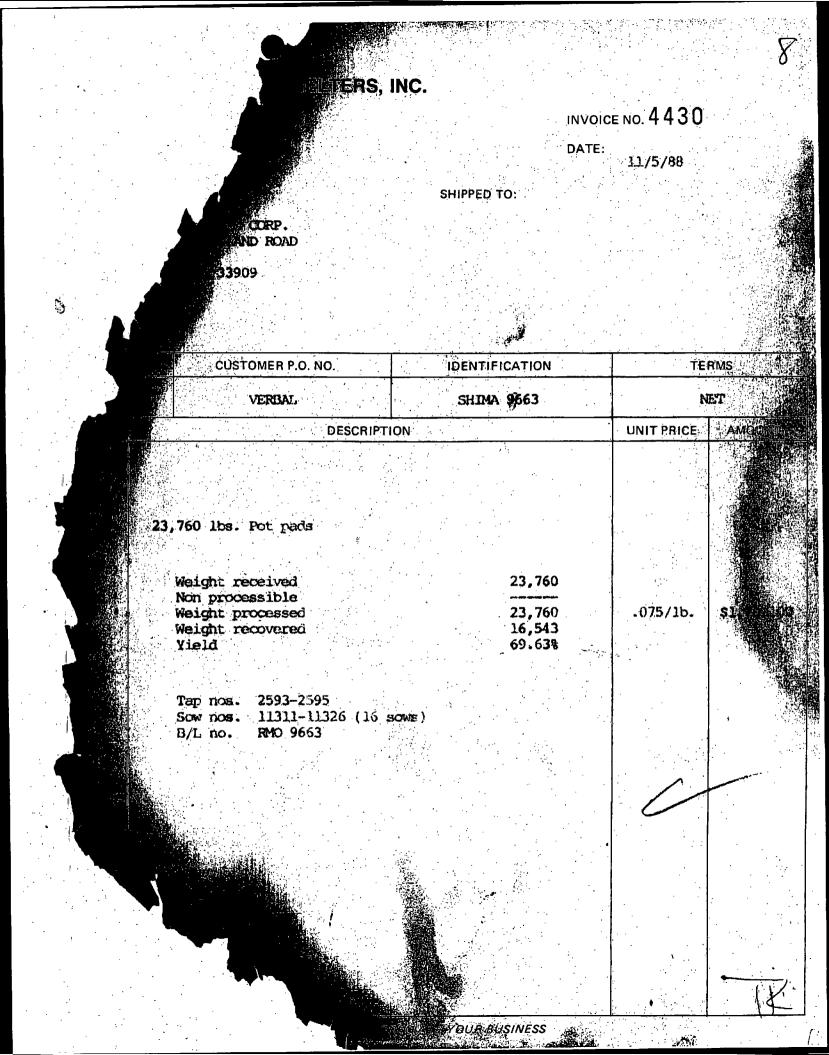


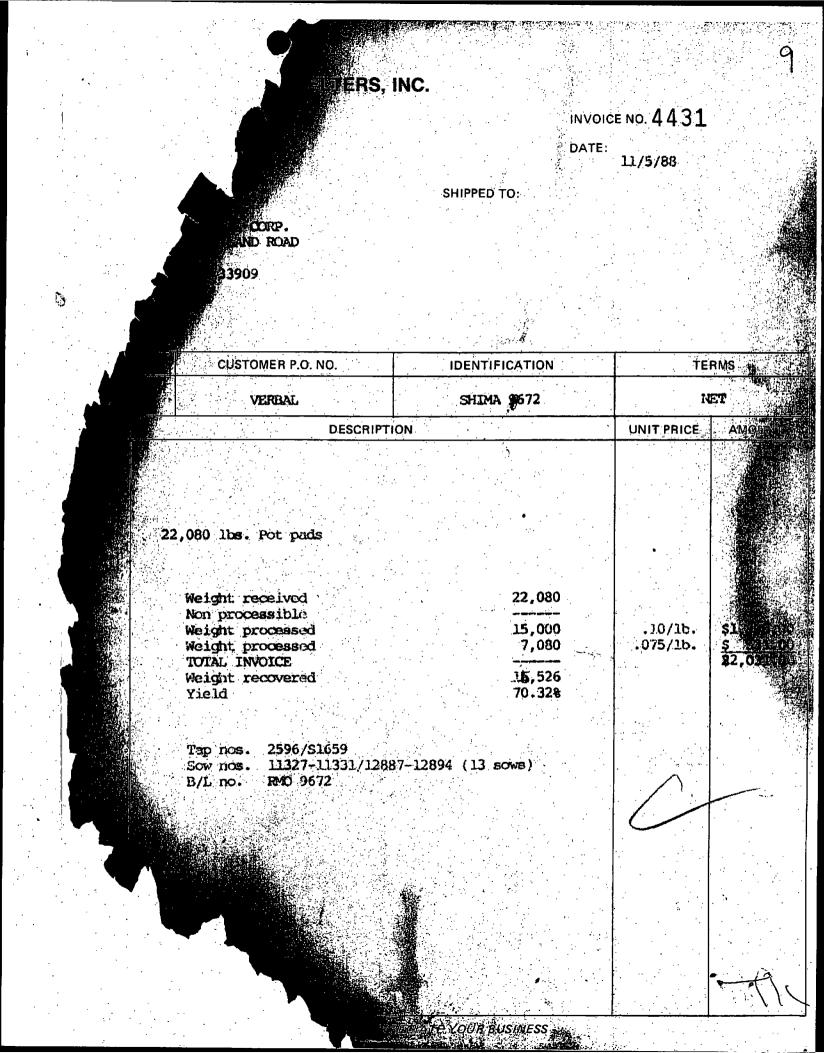




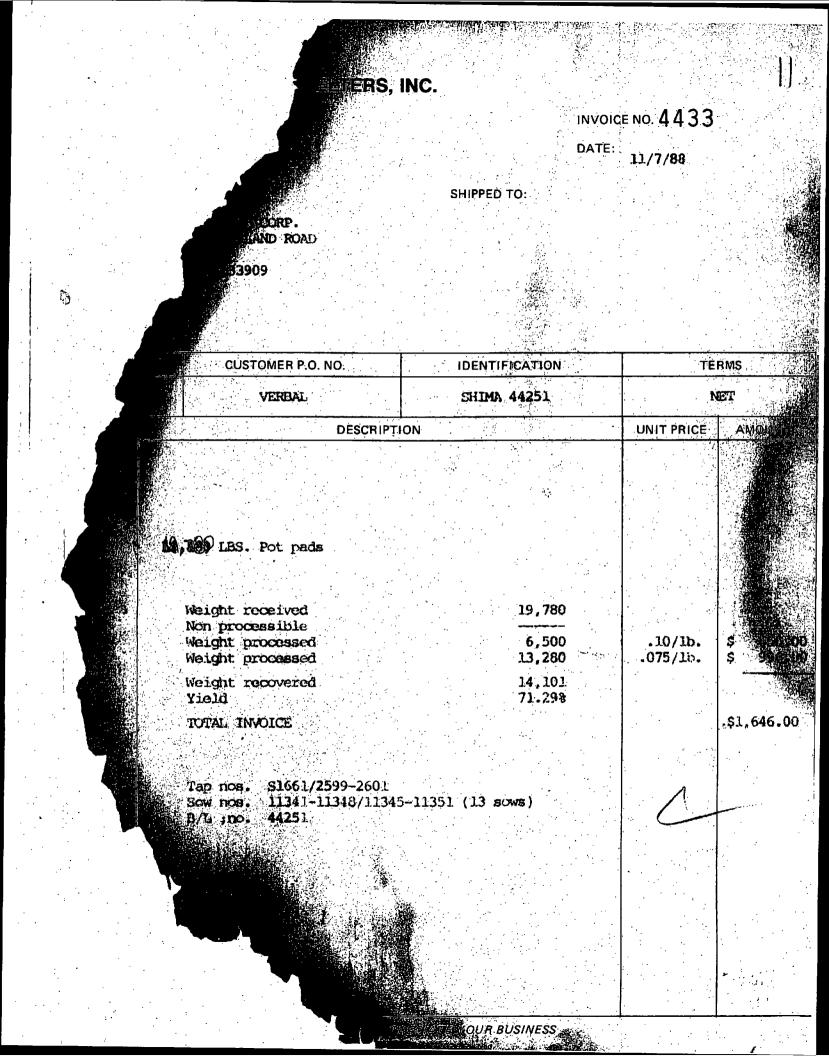


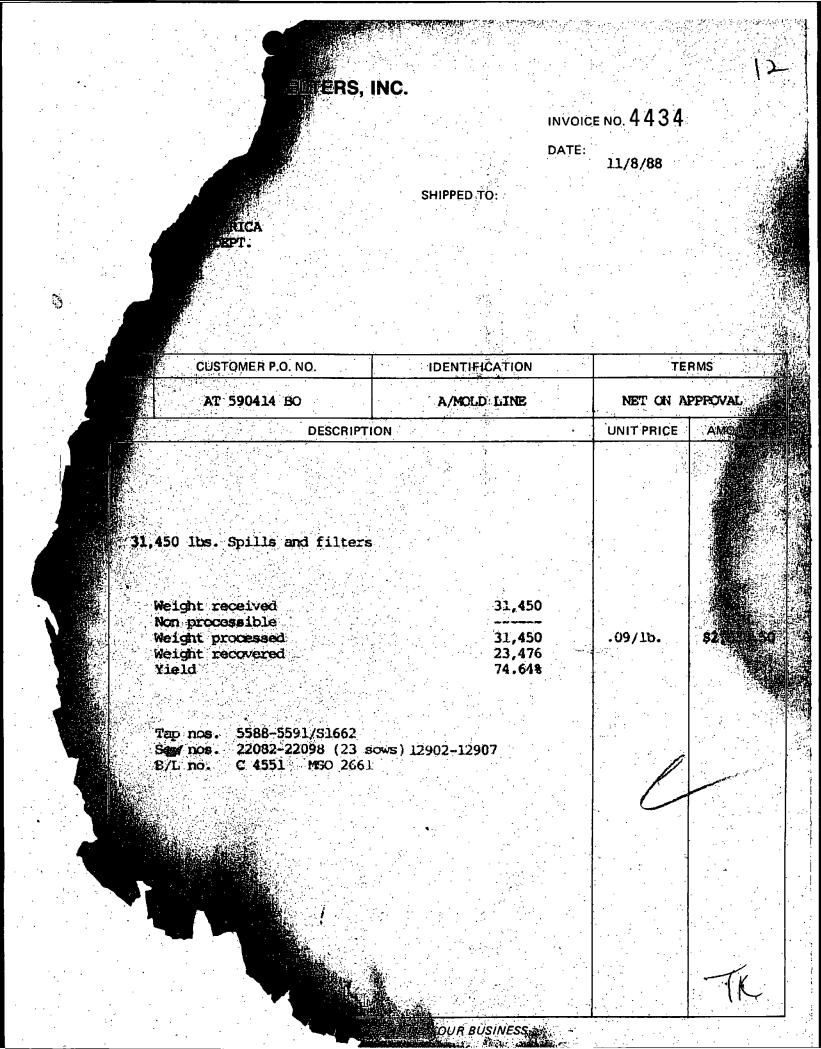


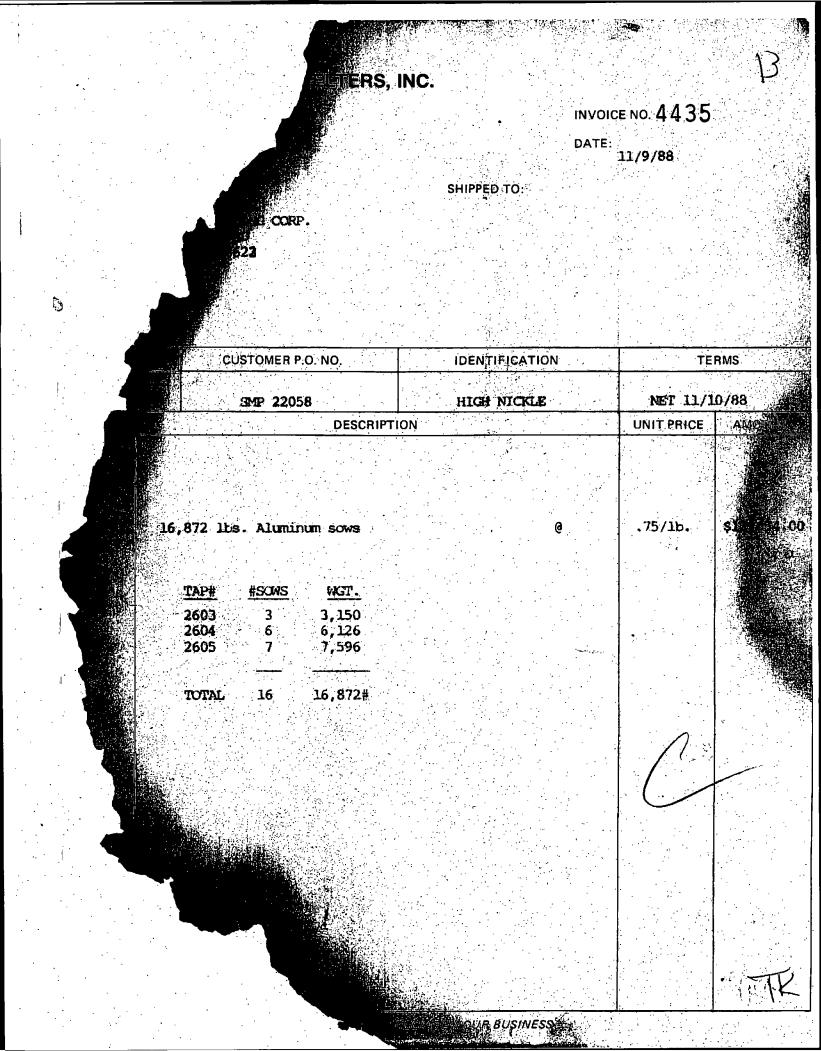




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			ICE NO. 4432	
		DATE		
			11/6/88	
		SHIPPED TO:		
	TORP.			
-	3909			
•			일하다. 열리 요설 - 100 - 111	
			1	
	CUSTOMER P.O. NO.	IDENTIFICATION	TERMS	
	VERBAL	SHIMA \$7716-1	NET	
	DESCRIPTION		UNIT PRICE AMO	
	20,760 lbs. Pot pads			
	Weight received	20,760		
	Non processible Weight processed	9,000	.10/Jb. \$ .075/1b. \$	
	Weight processed TOTAL INVOICE	11,760	\$1.7	
	Weight recovered Yield	15,788 76.05%		
				2 2
	Tap nos. S1660/2597-2598			
	Saw nos. 12895-12899/11332- B/L no. 57116-1	-11340 (14 sows)		
		WATE LOUR BUSINESS IN 18	1	
		A BUSINESS ILL	organismos programas de la companya	Al and the second







ERS	INC	14
	•	ICE NO. <b>4436</b>
	INVOI	
	DATE	11/9/88
	SHIPPED TO:	
CUSTOMER P.O. NO.	IDENTIFICATION	TERMS
AT 161248 CN	C-PAN	NET ON APPROVAL
DESCRI		UNIT PRICE AMOUNT
Weight received Non processible Weight processed Weight recovered Yield	16,590 16,590 10,995 66.278	.10/lb. <b>\$1,</b> 10
Tap nos. S1663 Sow nos. 12908-12917 (10 B/L no. MSO 3620	0 sows)	

				15
	A STERS, I			
			DICE NO. 4437	
		DATI	E: 11/9/88	
		SHIPPED TO:		
	a Car			
9				
	CUSTOMER P.O. NO.	IDENTIFICATION	TE	RMS
	AT 161248 CN	e-pan	net on api	PROVAL
	DESCRIPTION		UNIT PRICE	AMOU
	16,490 lbs. Crushed material			
		15 400		
	Weight received Non processibleiron, dust	16,490 . etc1,450	.14/lb.	
	Weight processed Weight recovered	15,040 6,611 43.96%	*14/.10	\$ <b>2</b> -)}
	Yield	33,300		
	Tap nos. 2609			
	Tap nos. 2609 Sow nos. 11386-11391 (6 so B/L no. MSO 3622	ws)		
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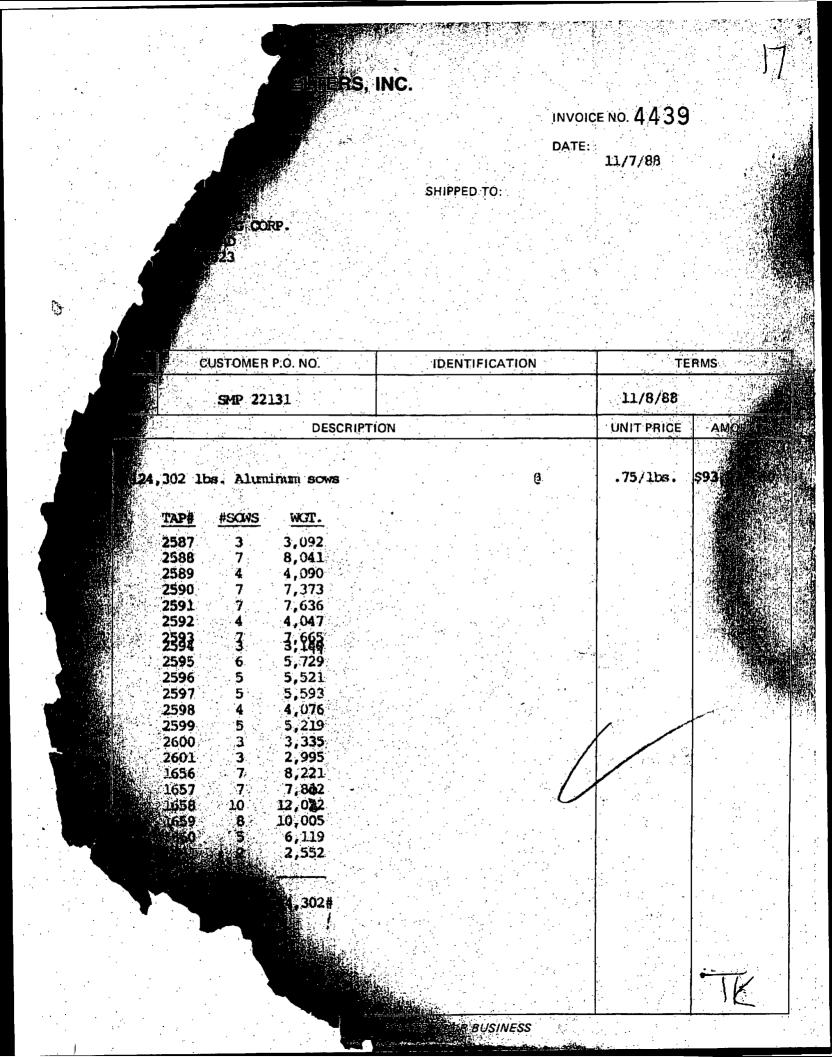
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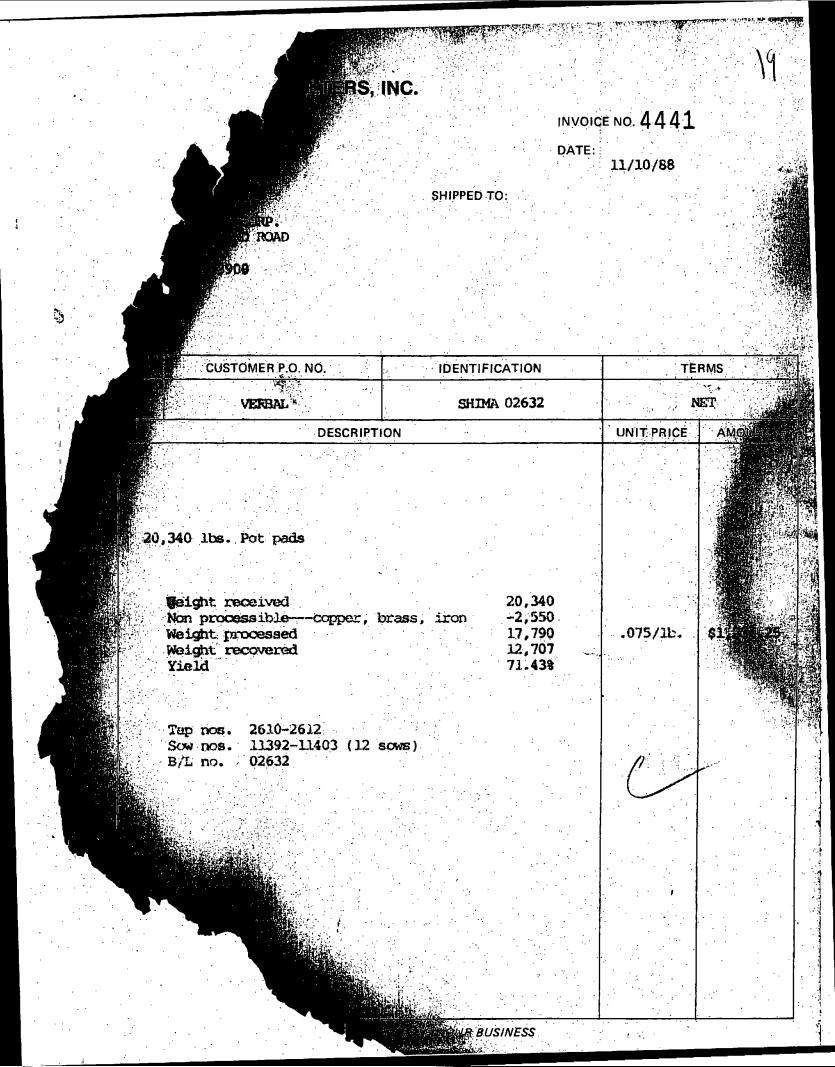
DATE:

11/9/88

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	CUSTOMER P.O. NO.	IDENTIFICATION	TERMS	
	VERBAL	DWPP	NET 30 DAYS	116
12.0	DESCRI	PTION	UNIT PRICE A	10
	8,180 lbs. Solids and spills Weight received	8,180		
	Non processible Weight processed Weight recovered Yield	8,180 5,715 69.86%	.075/1b. <b>\$61</b>	
	Tap nos. 5592-5593 Sow nos. 22099-22103 (5 B/L no. 11107	sows)		
				•



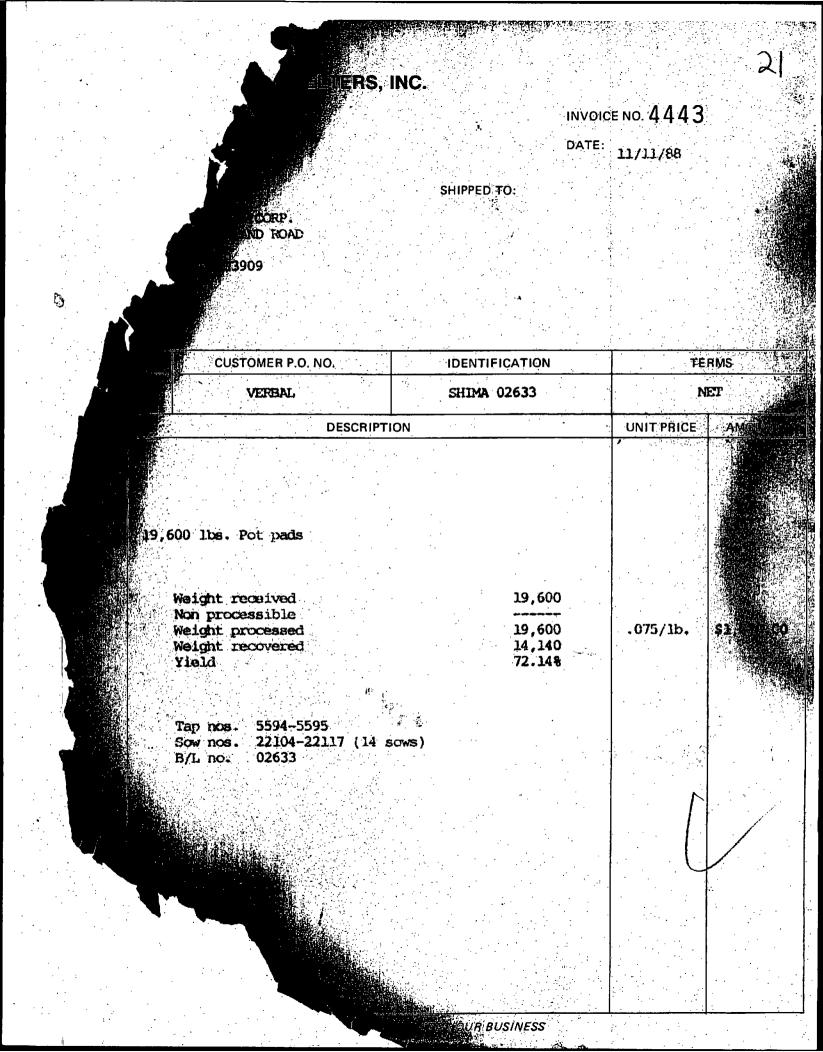


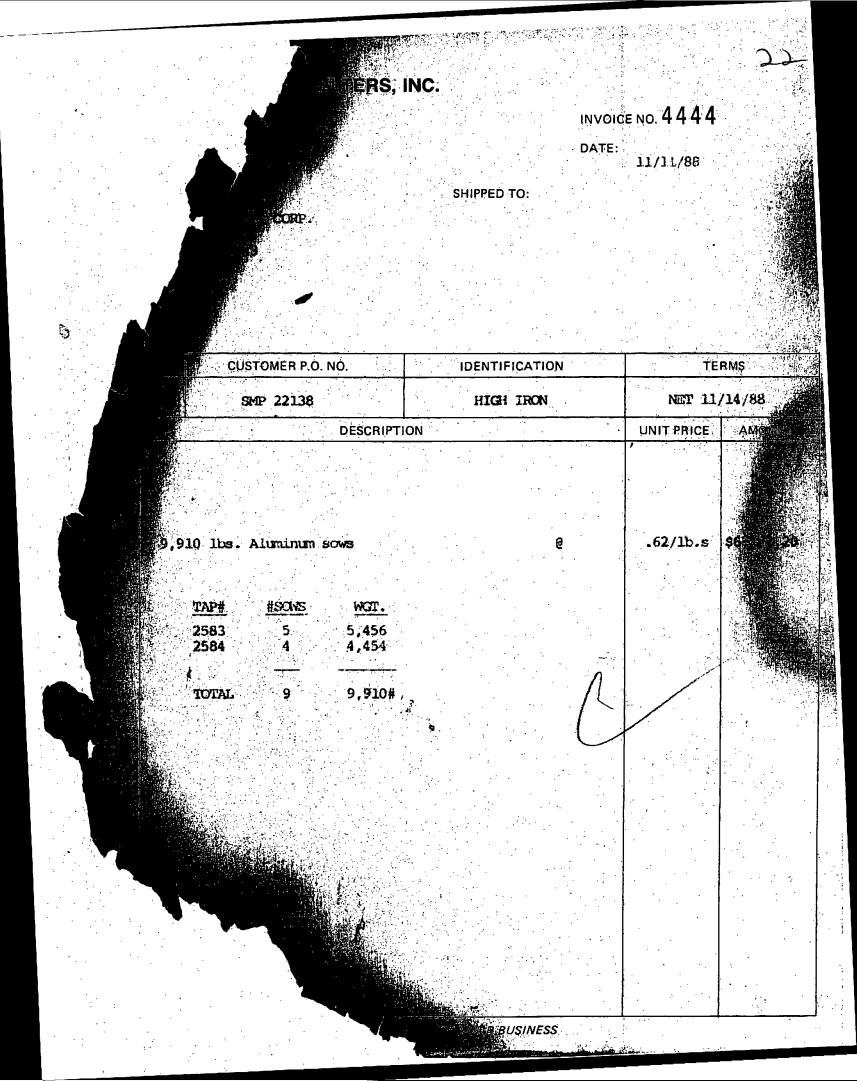
ERS, INC.

INVOICE NO. 4442

DATE: 11/11/88

	CUSTOMER P.O. NO. IDENTIFICATION	TE	RMS
4	VERBAL SHIMA 02634	li vala	VET
	DESCRIPTION	UNIT PRICE	AMO
	9,780 lbs. Pot pads		
	3,700 lbs. For paus		
	20.700		
	Weight received 19,780 Non processible		
	Weight processed 19,780	.075/lb.	\$1.4
	Weight recovered 13,356		
	Yield 67.52%		
	Tap nos. 2613-2614		
	Sow nos. 11404-11415 (12 sows) B/L no. 02634		1
	2 15/11 110;		
	AL SOUTH	-	





ERS, INC.

DESCRIPTION

SHIPPED TO:

ND ROAD

CUSTOMER P.O. NO.

VERBAL

20,240 lbs. Pot pads

Weight received

Non processible Weight processed

Weight processed TOTAL INVOICE

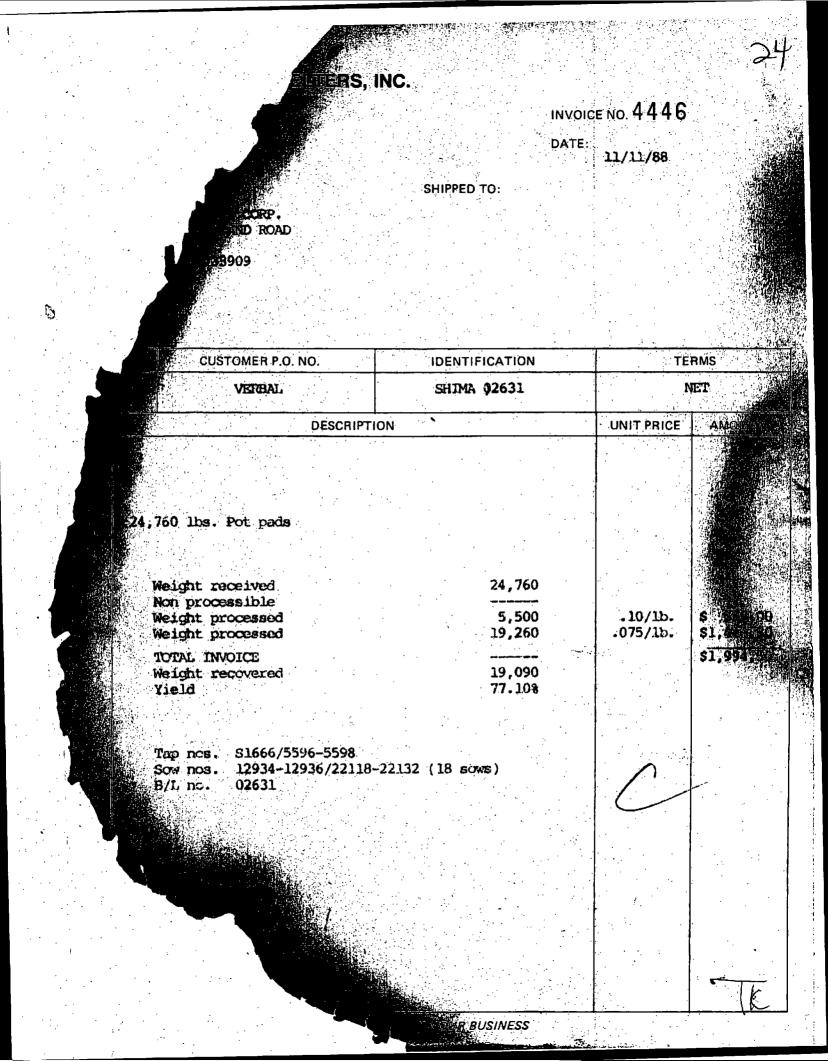
Weight recovered Yield :

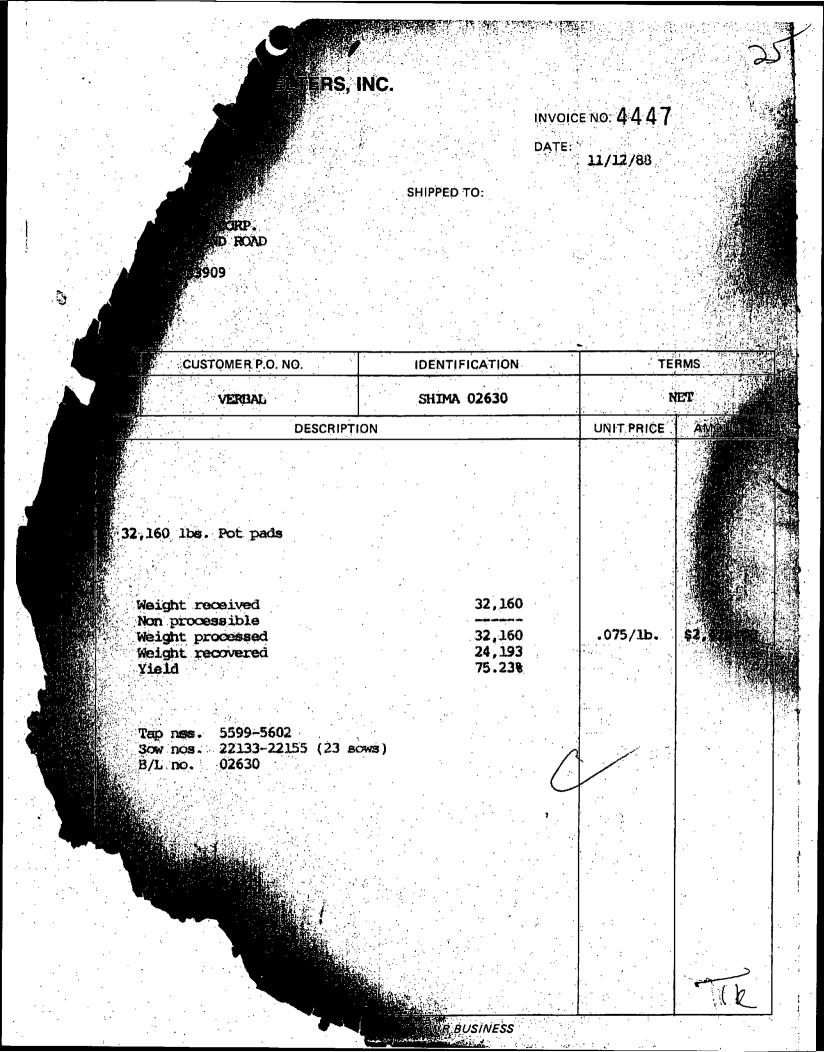
Tap nos. \$1665/2615

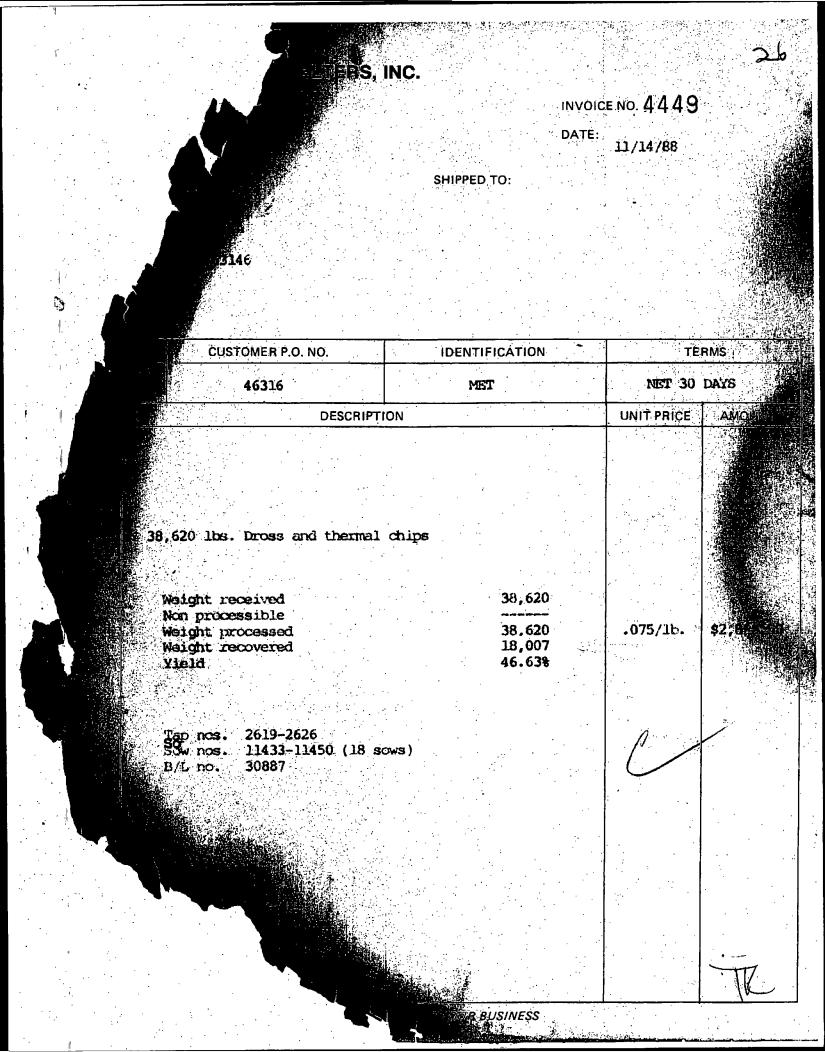
Sow nos.

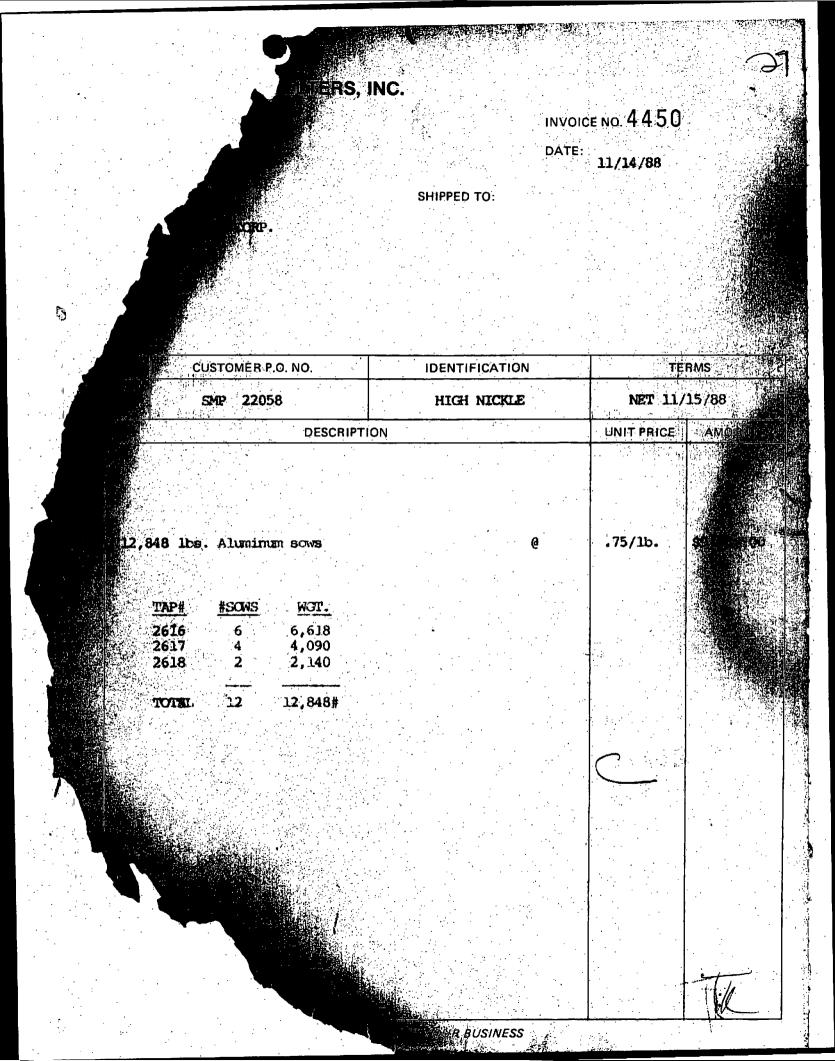
12926-12933/11416-11420 (13 sows)

B/L no. 02635



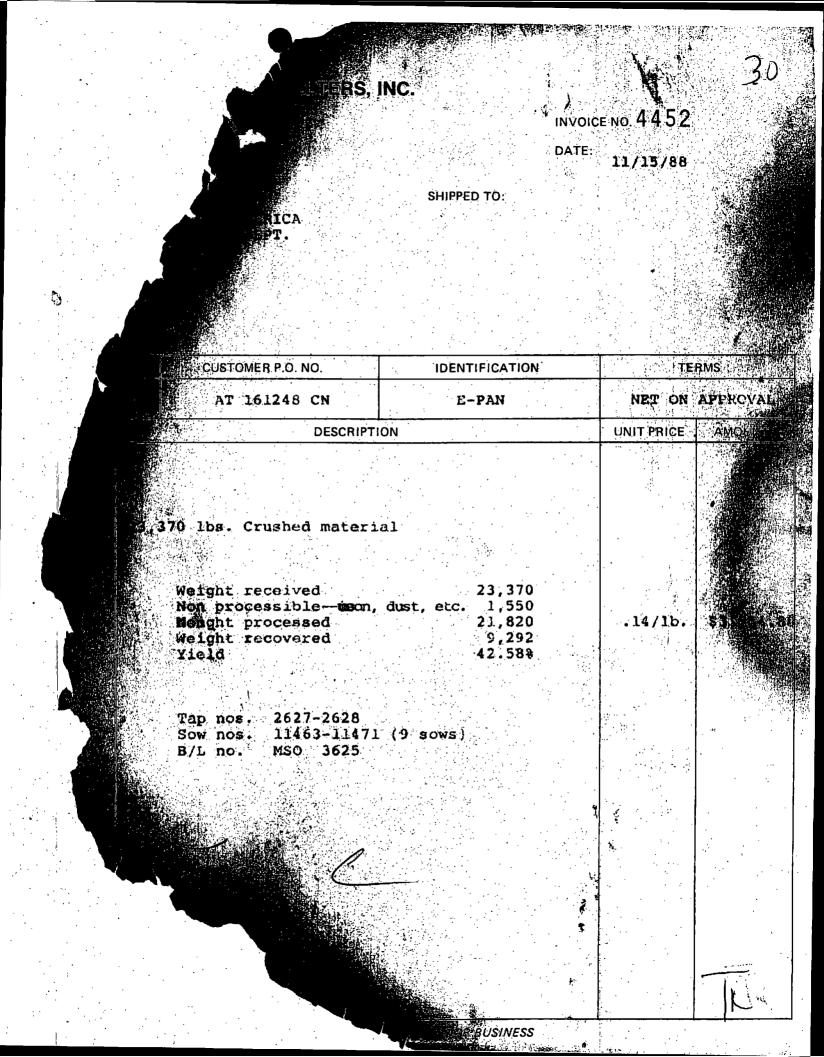




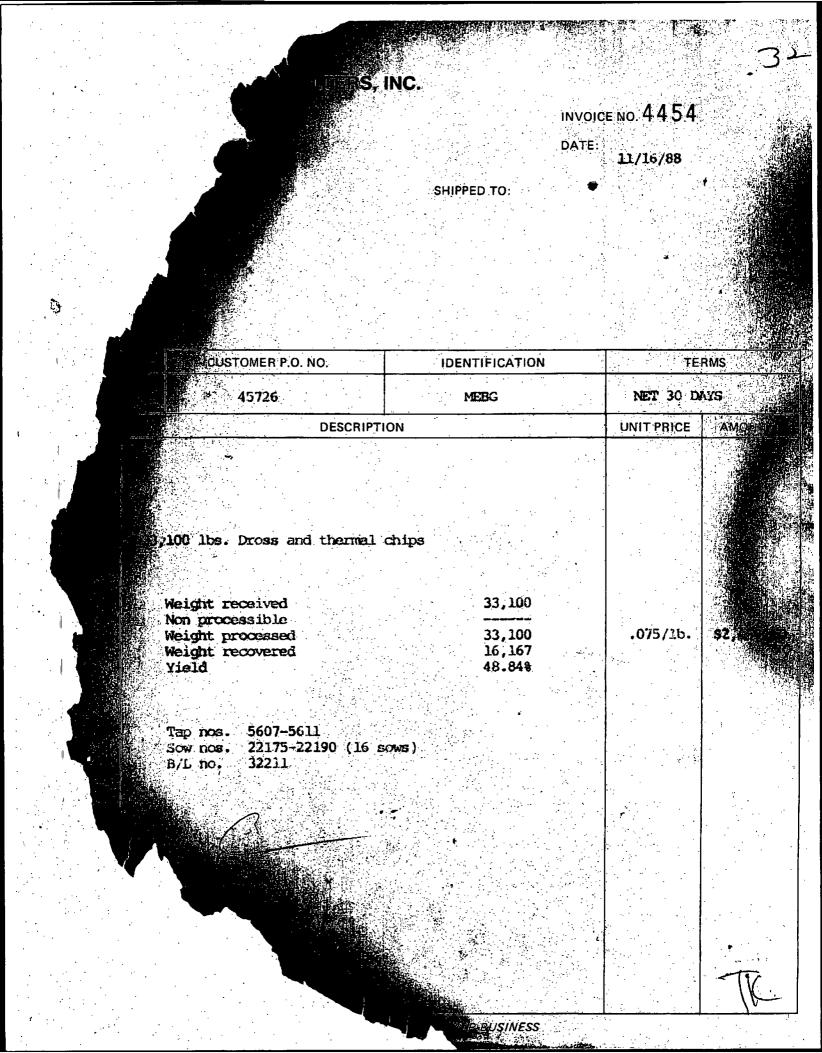


			$\mathcal{A}$
ERS, II		E NO. 4448	
	DATE:	11/14/ <b>8</b> 8	
CUSTOMER P.O. NO.	IDENTIFICATION		RMS
VERBAL	A-1	net on Re	CEIPT
DESCRIPTIO	N	UNIT PRICE	AMOUT 17
Weight received  New processible  Weight processed  Weight recovered  Yield	35,245 	.075/1b.	S2
Tep nos. 5603-5606 Sow nos. 22156-22174 (19 so B/L no. 5350/5338/5351	JWB)		

	RS, INC			29
			OICE NO. <b>4451</b> TE: 11/15/88	
CUSTOME	≅R P.O. NO.	IDENTIFICATION		
	PEAL.	so. Tlux	NET ON H	
	DESCRIPTION		UNIT PRICE	AMO
16,650 lbs. One Weight rece Non process Weight proce Weight reco Yield	ived ible essed vered	16,650 16,650 8,336 50.078	.075/16.	
Sow nos. 1	625-2626 1455-11462 (8 sows 0 #			
		្ទុប់គូ BUSINESS		I.T.

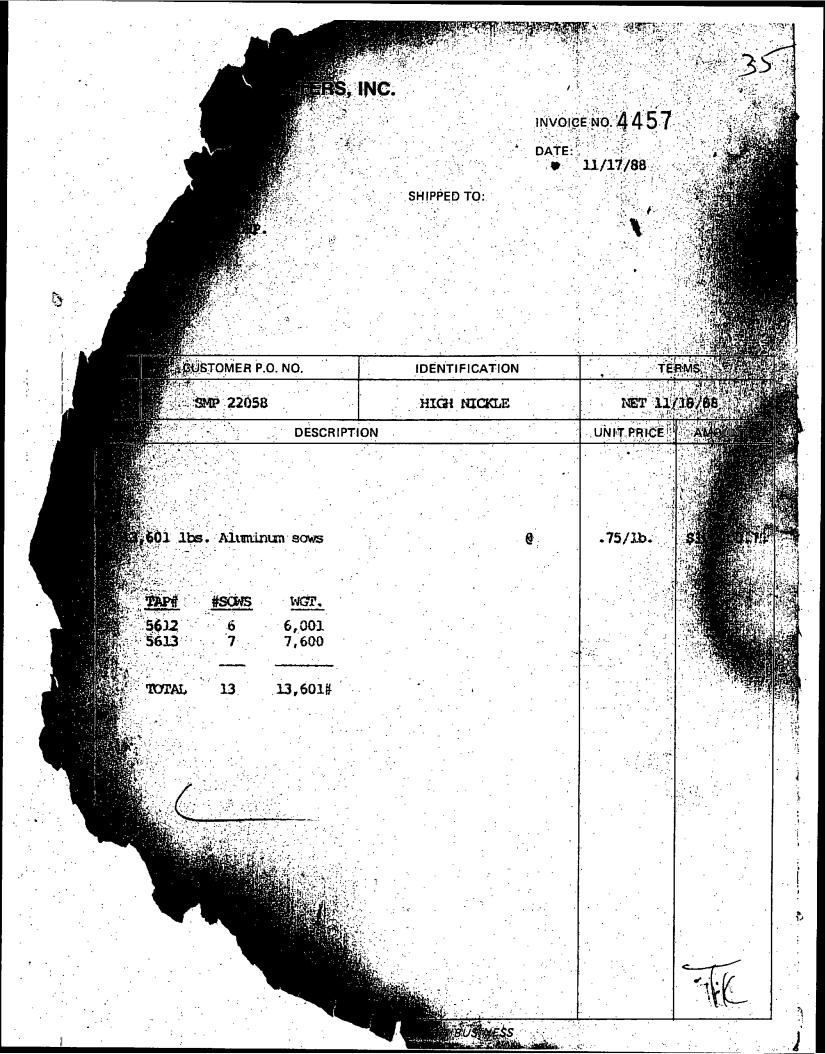


	ZS, IN			3/
			INVOICE NO. 445 3 DATE: 11/16/88	
		SHIPPED TO:	11/16/88	
CUSTOMER P.O. N		IDENTIFICATION		RMS
AT 590414 BO		MOLD LINE		VPPROVAL)
	DESCRIPTIO	N	UNIT PRICE	AMOU
19,600 lbs. Mold line Weight received Non processible	scrap	19,600		
Weight processed Weight recovered		19,600 14,438	.09/lb.	
Tap nos. S1667-S. Sow nos. 12937-13 B/L no. C 4571	2948 (12 so	73.66% ows)		
				Tile
		III) FINE INESS		

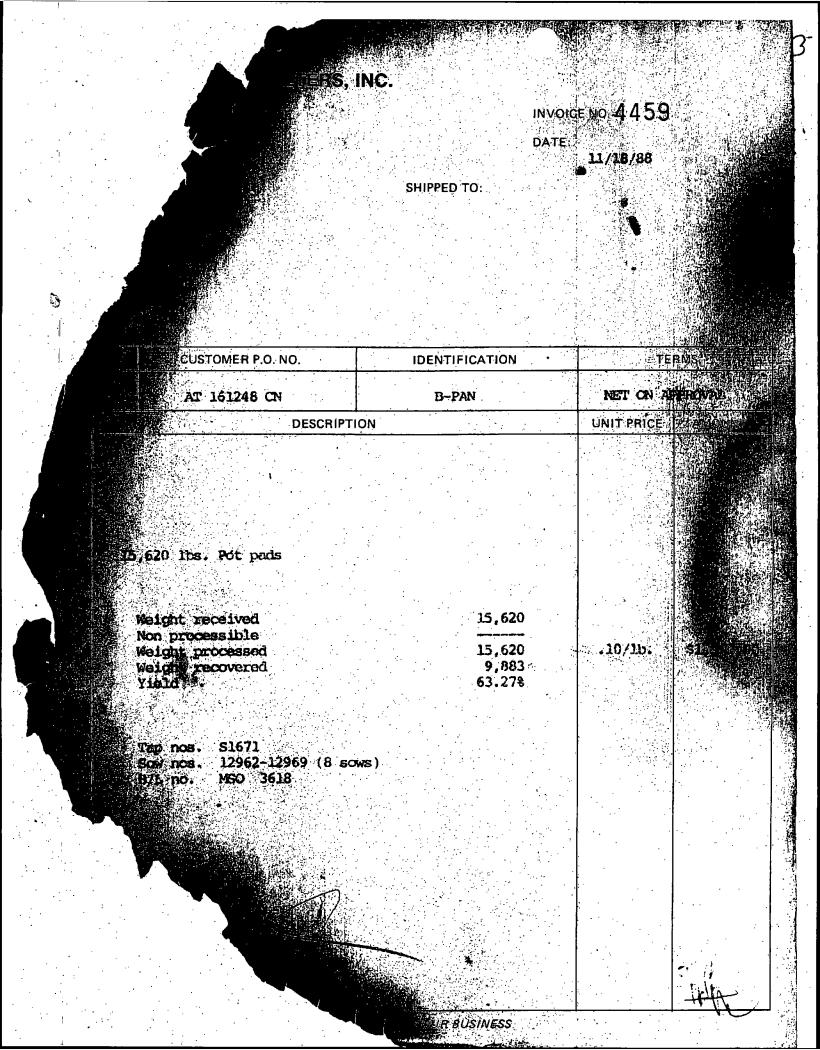


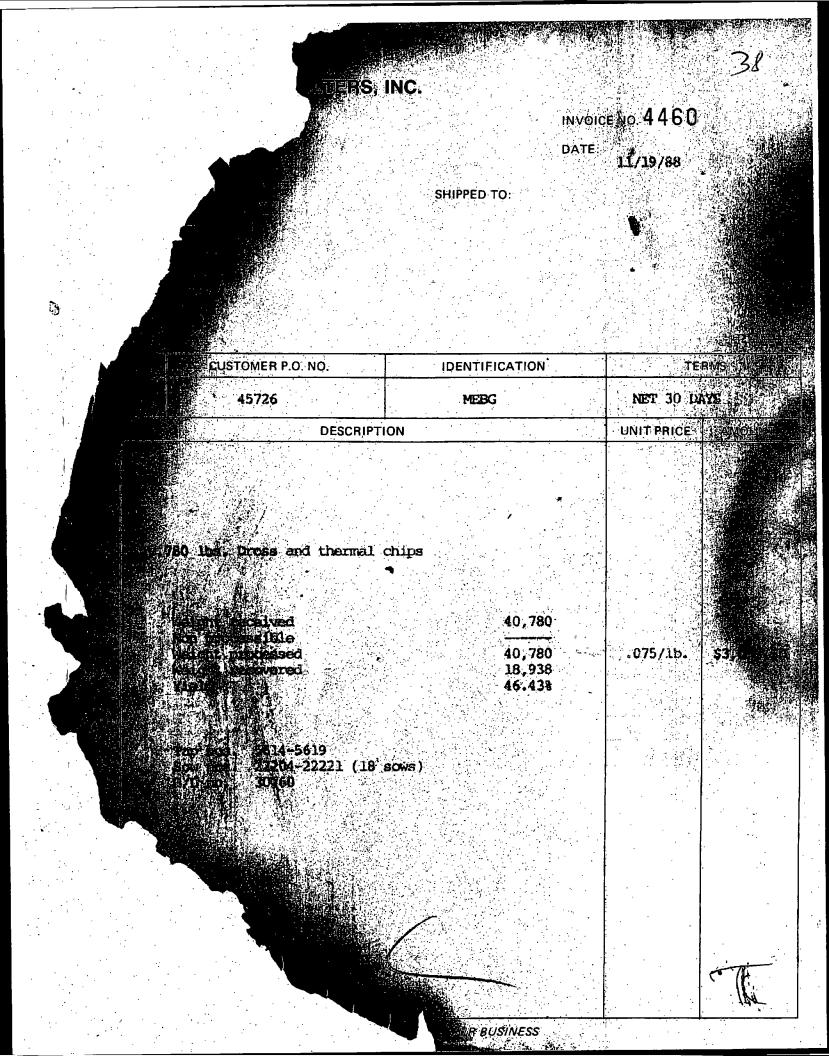
CUSTOMER P.O. NO.  SMP 22147  DESCRIPTION	SHIPPED TO:	DATE	NET 11/1	RMS 7/88
SMP 22147	IDENTIFICATION		NET 11/1	
SMP 22147	IDENTIFICATION		NET 11/1	
SMP 22147			NET 11/1	
SMP 22147		<b>\</b>	NET 11/1	
SMP 22147			NET 11/1	
SMP 22147		<b>v</b>	NET 11/1	
SMP 22147		<b>V</b>	NET 11/1	
				7/86
DESCRIPTION				
			UNITPRICE	AMOUN
192 lbs. Aluminum sows		a	.85/1b.	\$30
			103, 101	
3 3,294				
5 5,436				
<b>2615</b> 5 5,043				
5594 8 7,969 5595 6 6,171				
26 2024				
TOTAL 35 36,292#				
71,542 lbs. Aluminum sows		0	.78/1b.	\$55,802.76
TAP# #SQWS WGT. 1666 3 3,751				
5 4,498 7 7,547				1
6 6,468 5,879				
9.586 209				

A SECTION OF THE SECT		oice No. 4 4 5 6	34
	SHIPPED TO:	E: 11/17/88	
CUSTOMER P.O. NO.	IDENTIFICATION	TERM	12 (17 ) 2 (17
AT 590414`BO	MOLD LINE	NET CAN APP	ROVAL
DESCRIPTIO	ON	UNIT PRICE	AMOUNT
Waight received Non processible Weight processed Weight processed Yield	29,380 29,380 22,477 76,508	.09/lb.	
Tep nos. 2629-2631/S1669 Sow nos. 11472-11487/12949- B/L no. C 4570 MSO 2661	12953 (21sovs)		
	BUSINESS		NR.

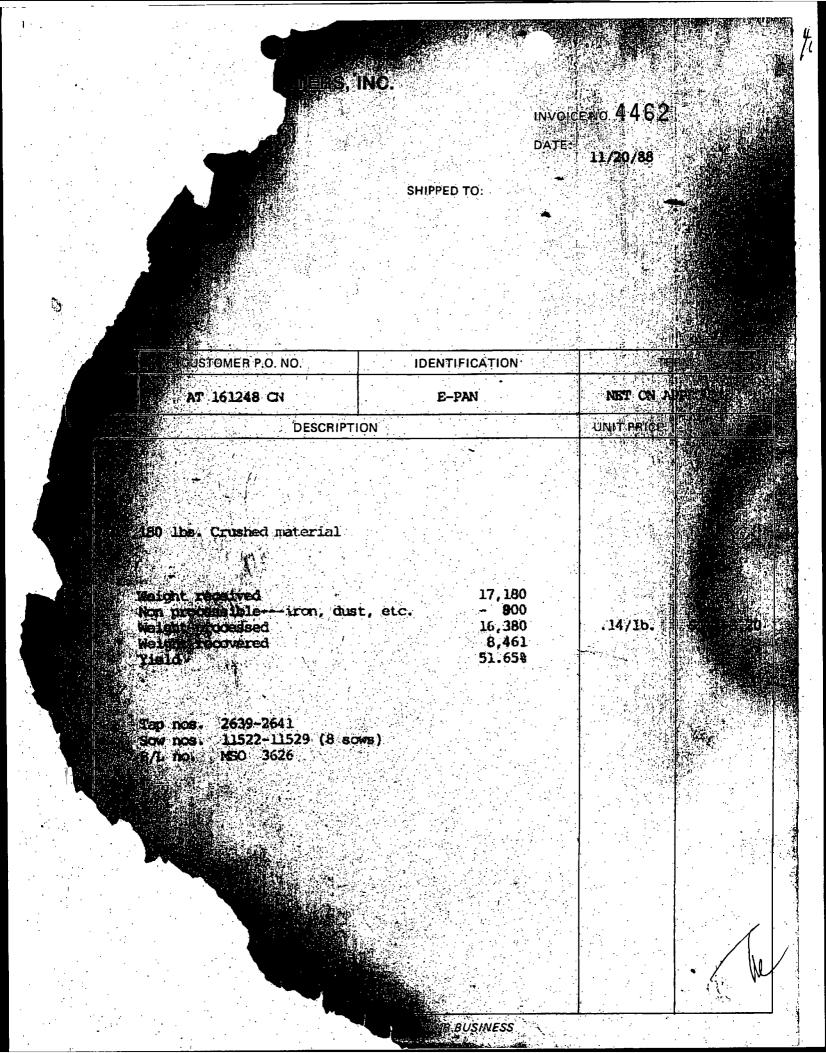


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	散作集 から 心に ピー・キュガン こがん	oice no. 4458	
	DA		
		11/17/88	
	SHIPPED TO:		
CUSTOMER P.O. NO.	IDENTIFICATION	TERMS	Mai .
AT 590414 BO	MOLD LINE	NET ON APPRO	Programme and
DESCRIPT	ION	UNIT PRICE	valeta Monna
Want 1			
90 lbs. Mold line scrap			
Weight received Non processible	33,890		
Weight processed Weight recovered	33,890 25,782 76.07%	.09/1b. 8	
/leld	76.07%		
Tap nos. 2632-2633/S1670 Sow nos. 11488-11503/1295	54-12961 (24 sows)		
B/L no. C 3200 MSO 26	61		
			•
		_	TE:
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	BUSINESS	<u> </u>	



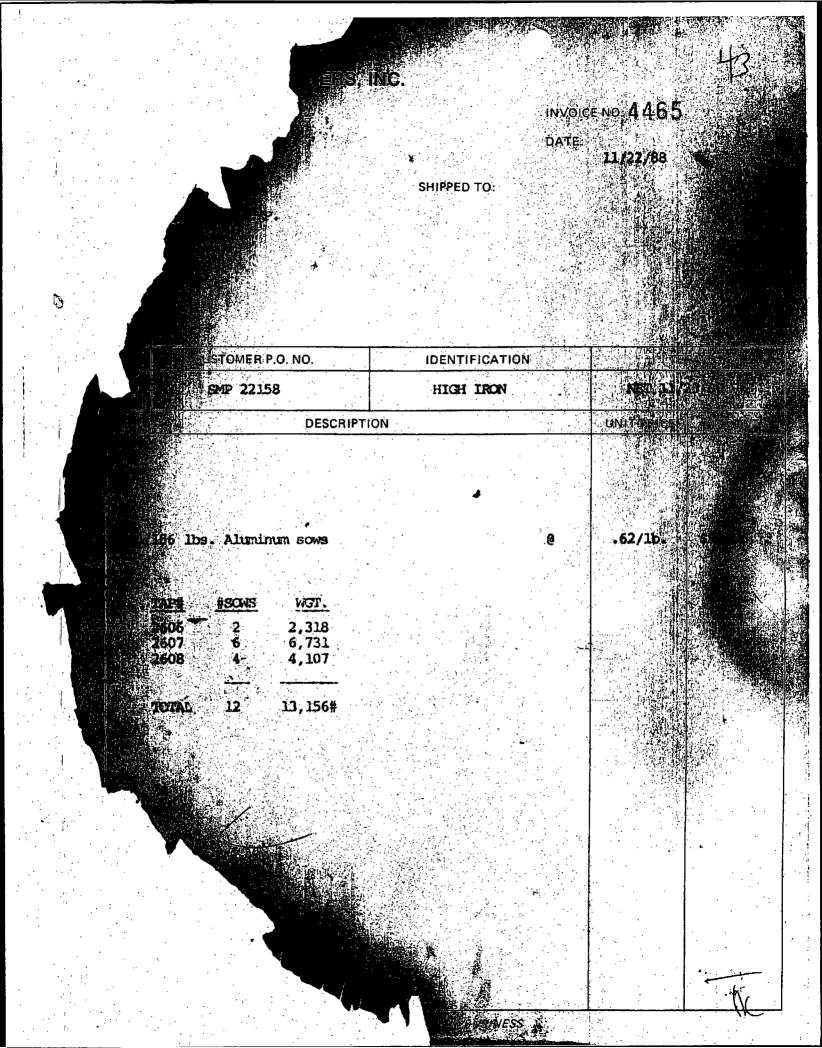


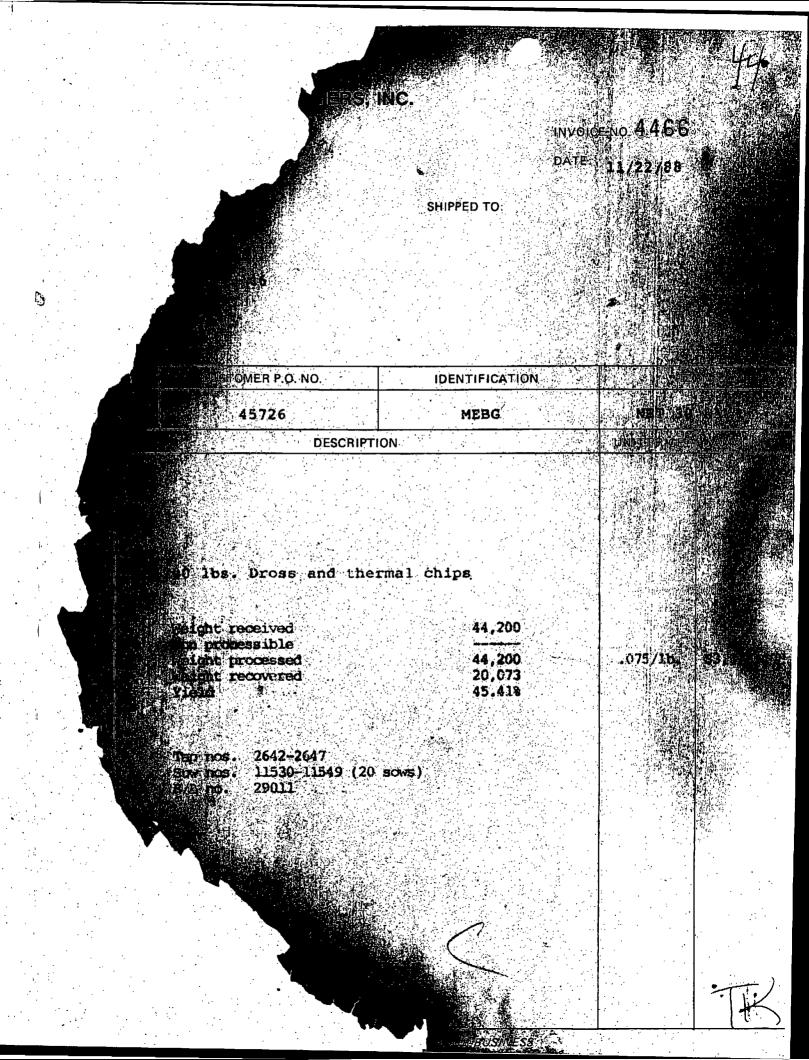
1				314
		INV	roiceNo.4461	
		SHIPPED TO:	TE 11/19/88	
	REGUSTOMER P.O. NO.	IDENTIFICATION	TENS	
	yerbal	EMERSON	NET	
	DESCRIPTIO	DN	UNIT PRICE	
	020 lbs, Dross in drums			
	Weight Teceived Non purplessible Weight Trocessed Weight recovered Tield	29,920 	.40/16.	
4	Tap nos. 2635-2638 Sow nos. 11508-11521 (14 so			
		BUSINESS		

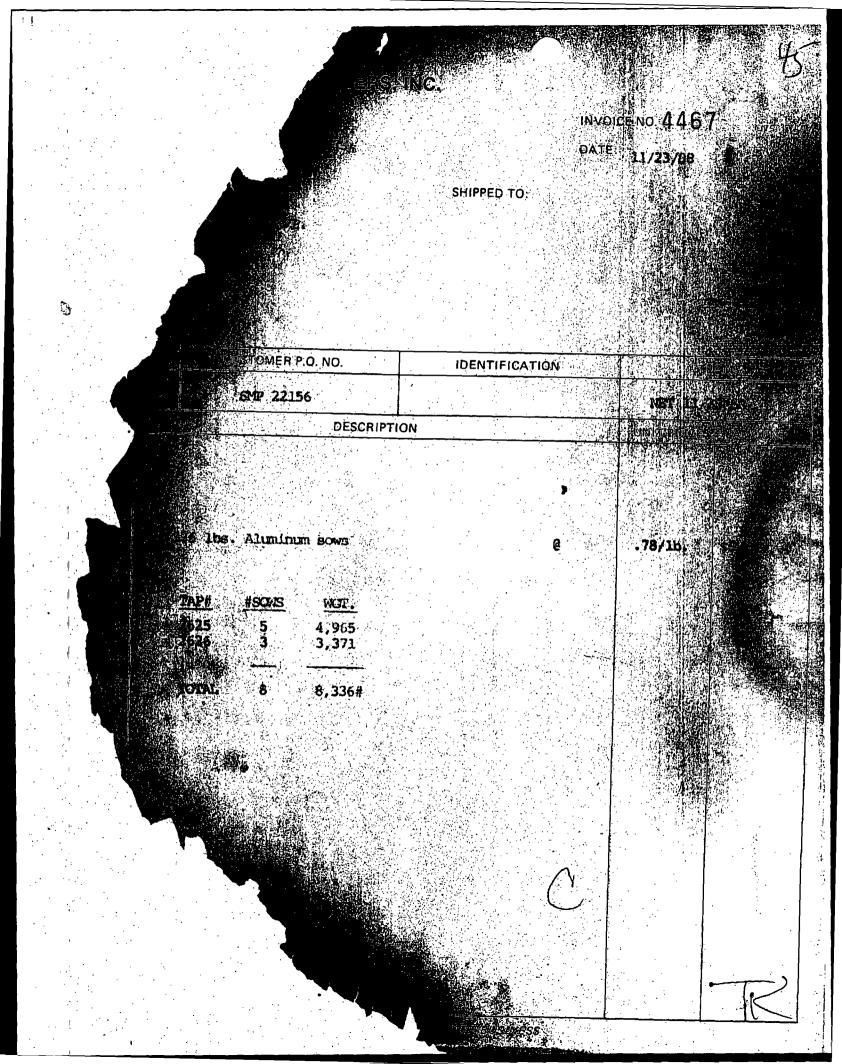


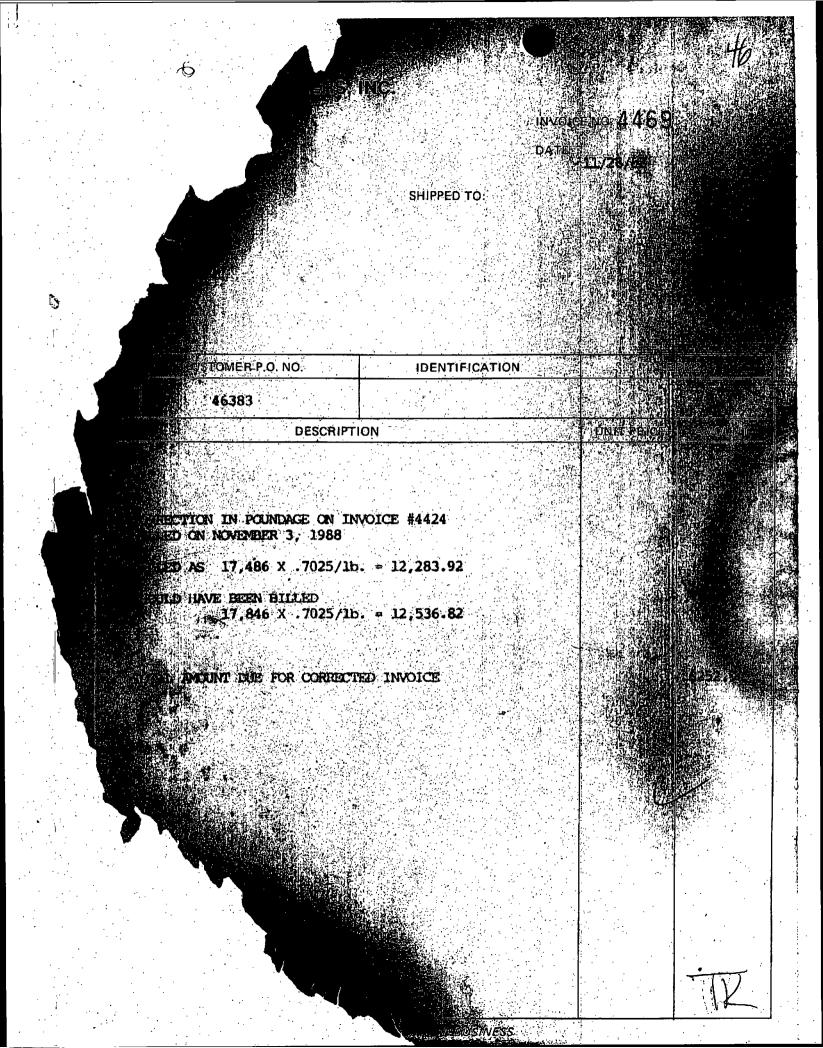
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		invoice no 446	3
		DATE	
		11/22/88	
	SHIPPED T <b>#</b> :		
USTOMER P.O. NO.	IDENTIFICATION	v in the second	arings of the second
AT 161248 CN	B-BOX	MEI O	
DESCRIPT		UNITERIO	A STATE OF THE STA
10.1bs. Pot pads		40 - 12 - 12 - 12 - 12 - 12 - 12 - 12 - 1	
maight received	48,1	30	
Weight processed	48,1	 <b>B</b> 0 .10/1b.	
Net processible Weight processed Weight recovered Visit	48,1 32,1 66.8	95 9 <del>8</del>	
150 nos. \$1672-\$1675			
Sew ness 12970-12996 (27 s // ness 04108 MSO 2259	sows)		
			1
			1
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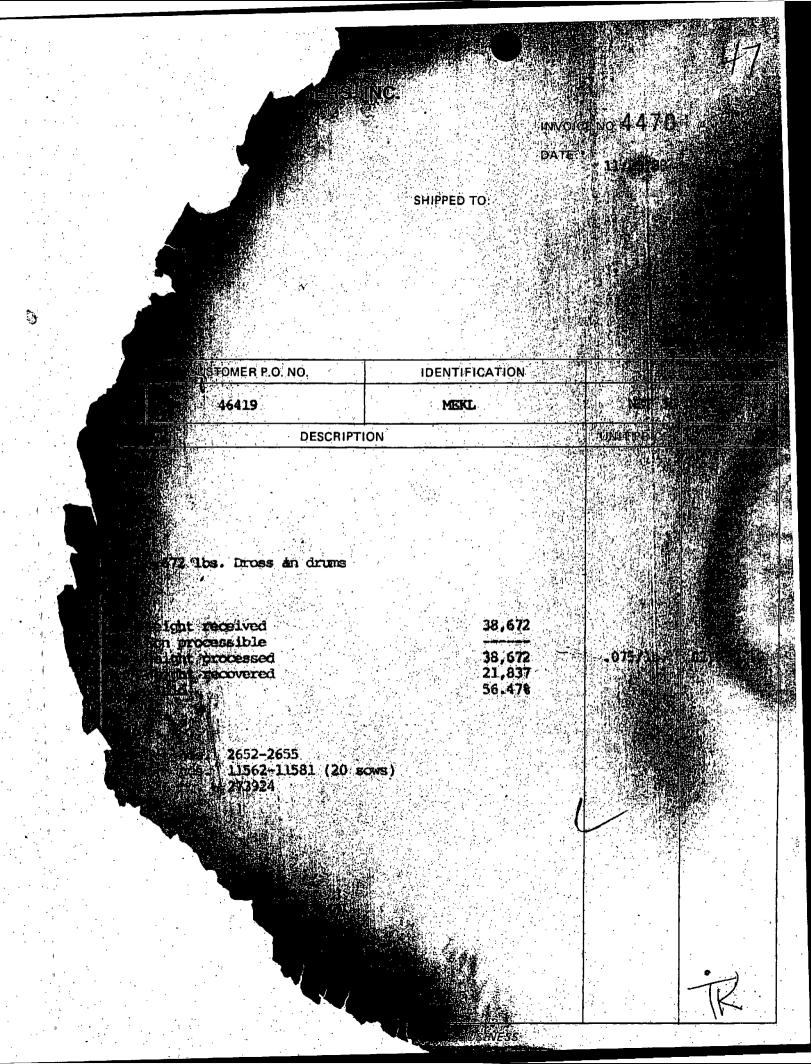
	i.v.	geng, 4464	
	DATE	<b>国际公司</b>	
	SHIPPED TO:		
STOMER P.O. NO.	IDENTIFICATION		
46448	INVENTORY TRANSPER		
DESCRIPT	ION	INTERIOR OF	
95 lbs. Aluminum sows	•	.90/lb.	
TAVE #SONS WGT.			
3834 7 4 4,405#			
		<b>大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大大</b>	
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	US/WESS.		

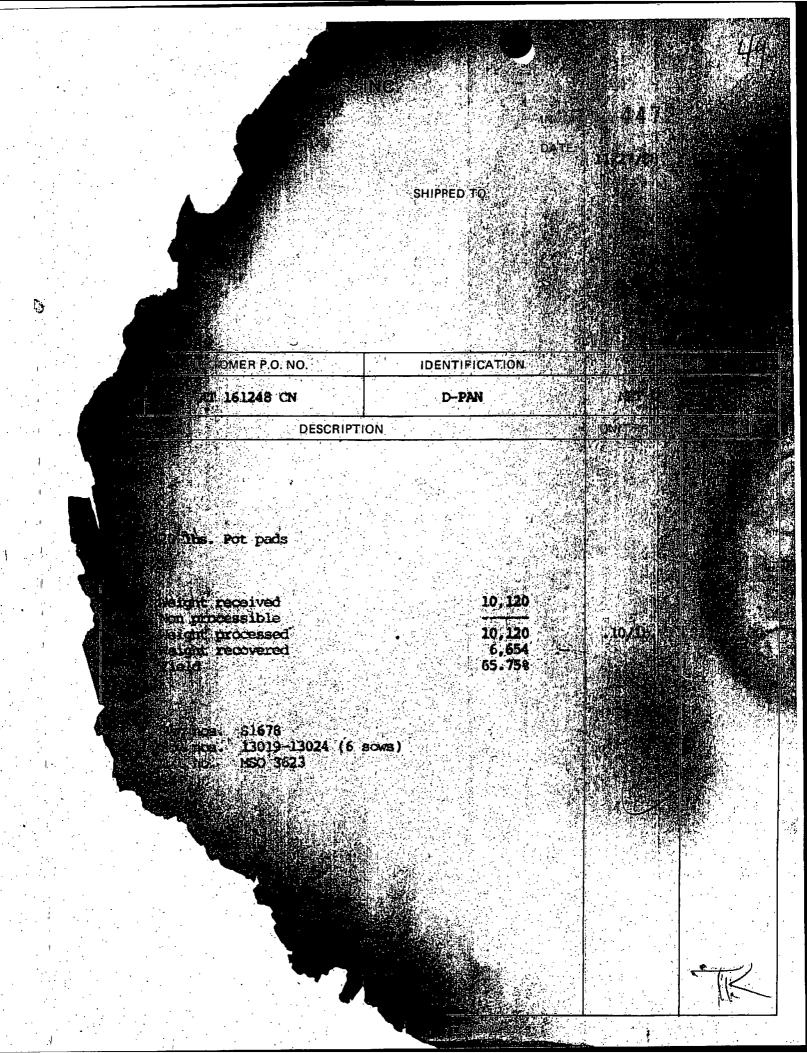


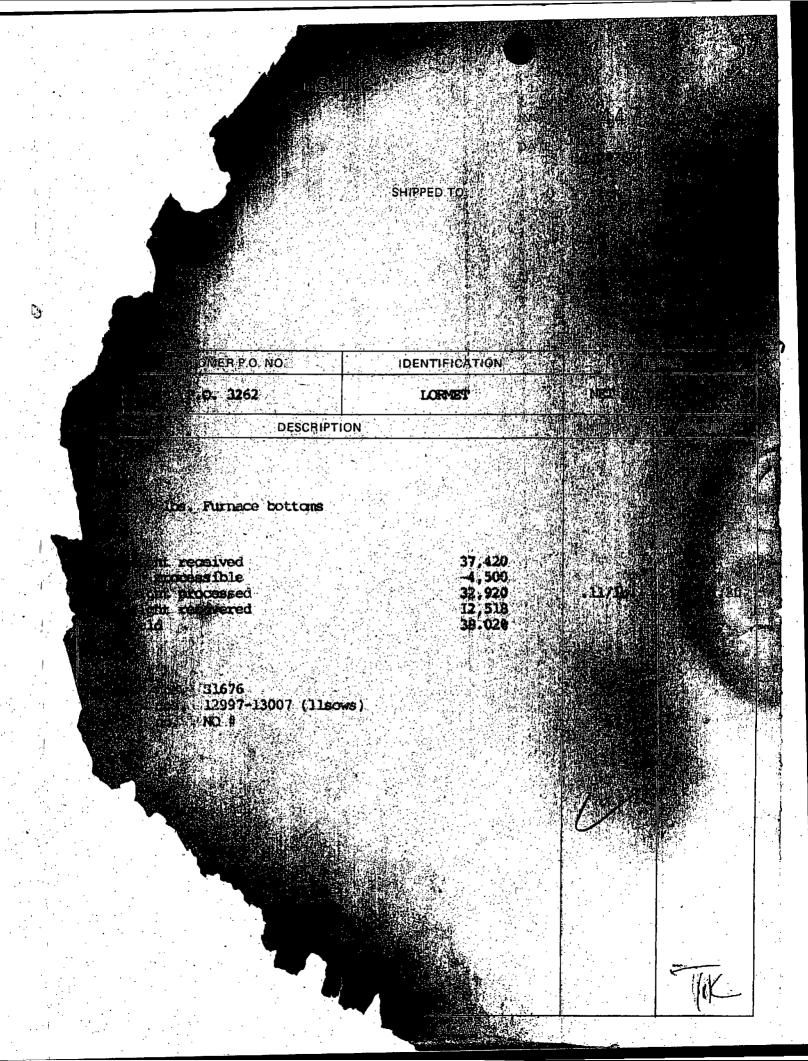


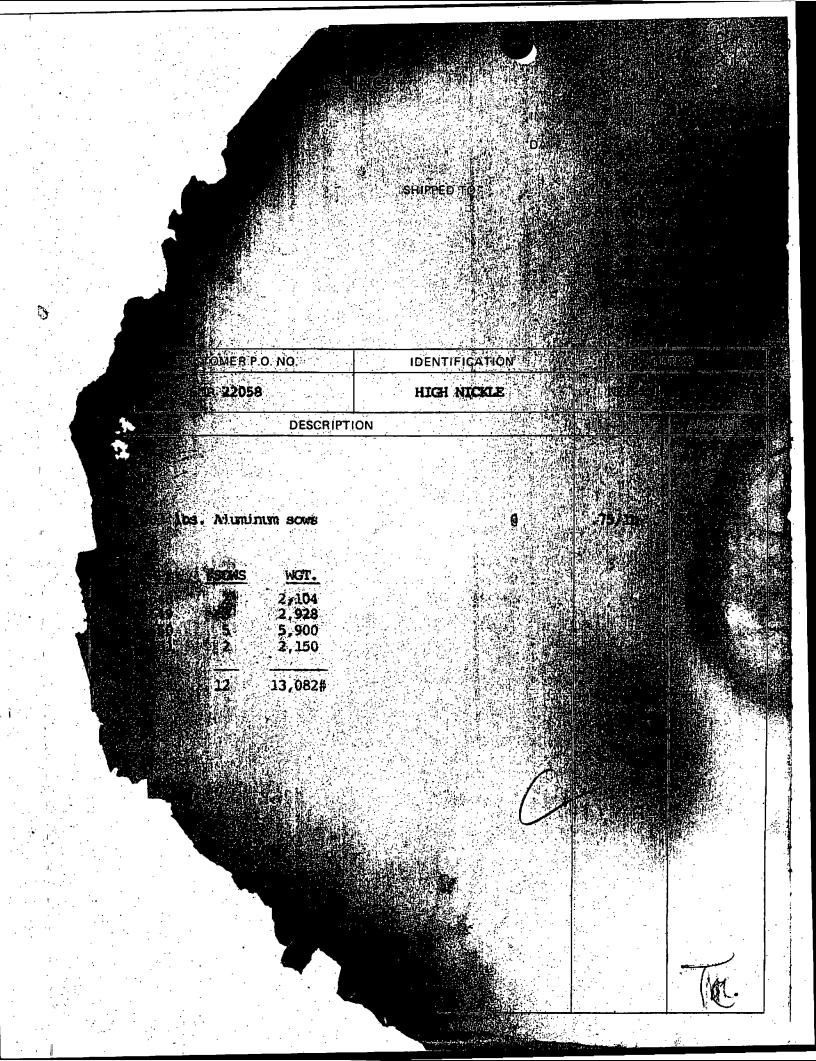


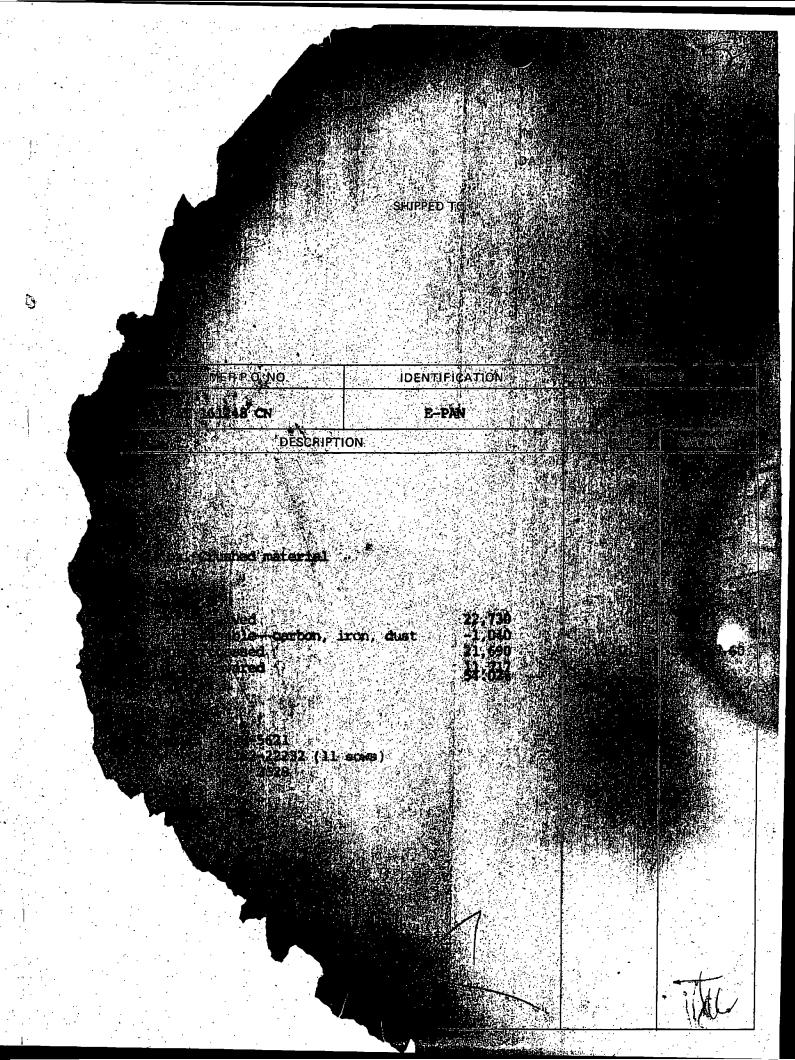


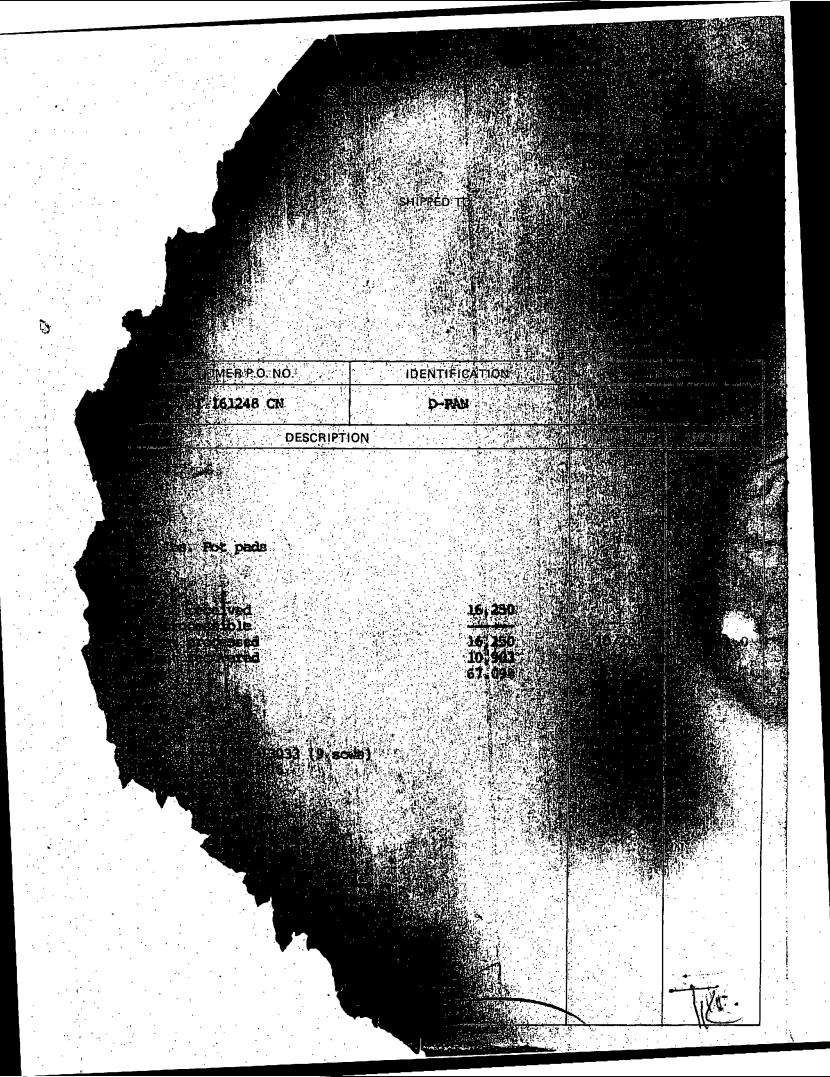


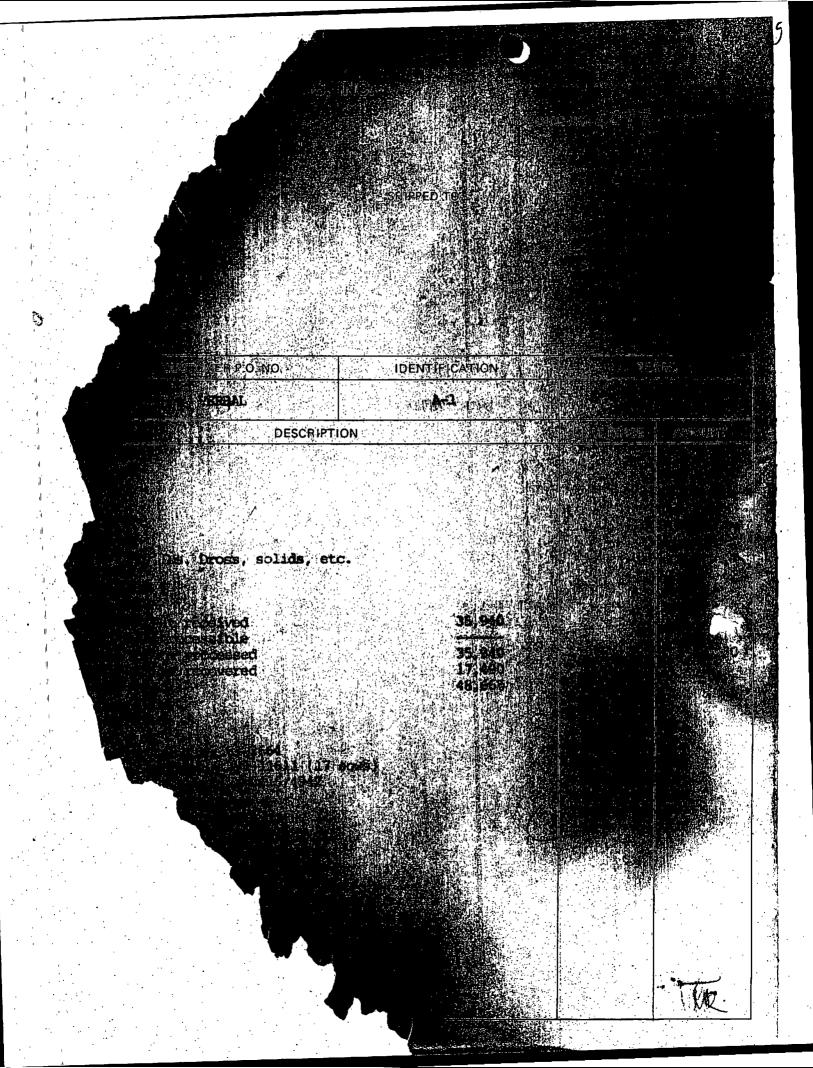


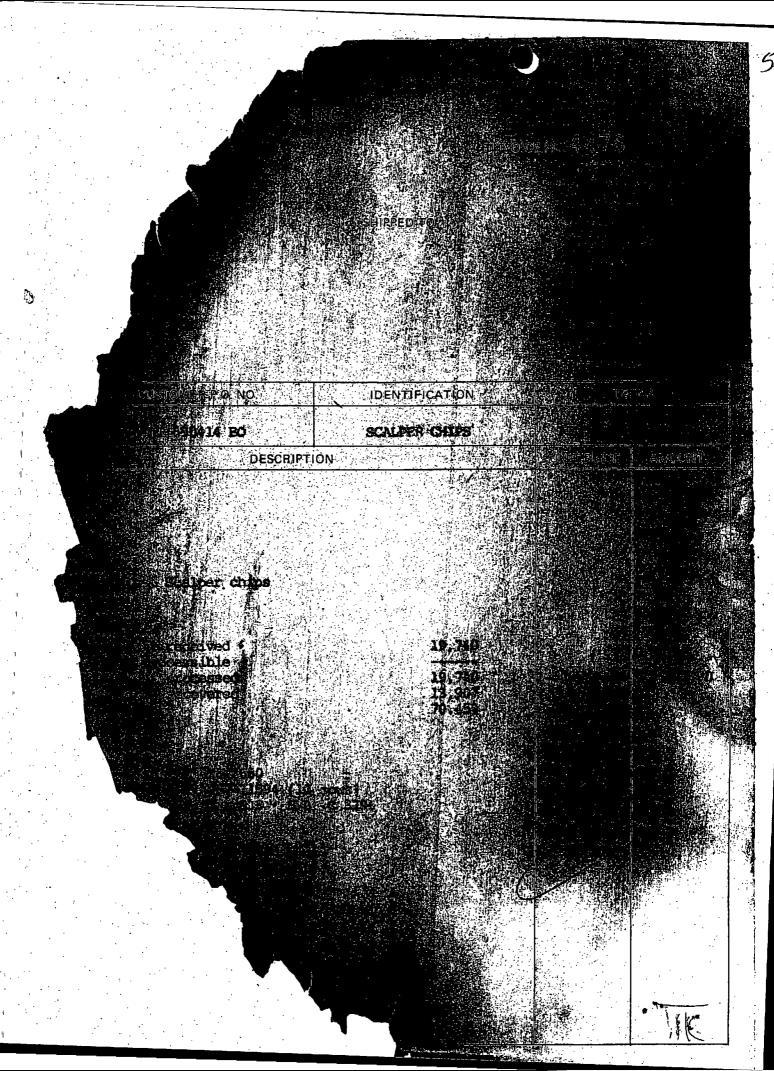


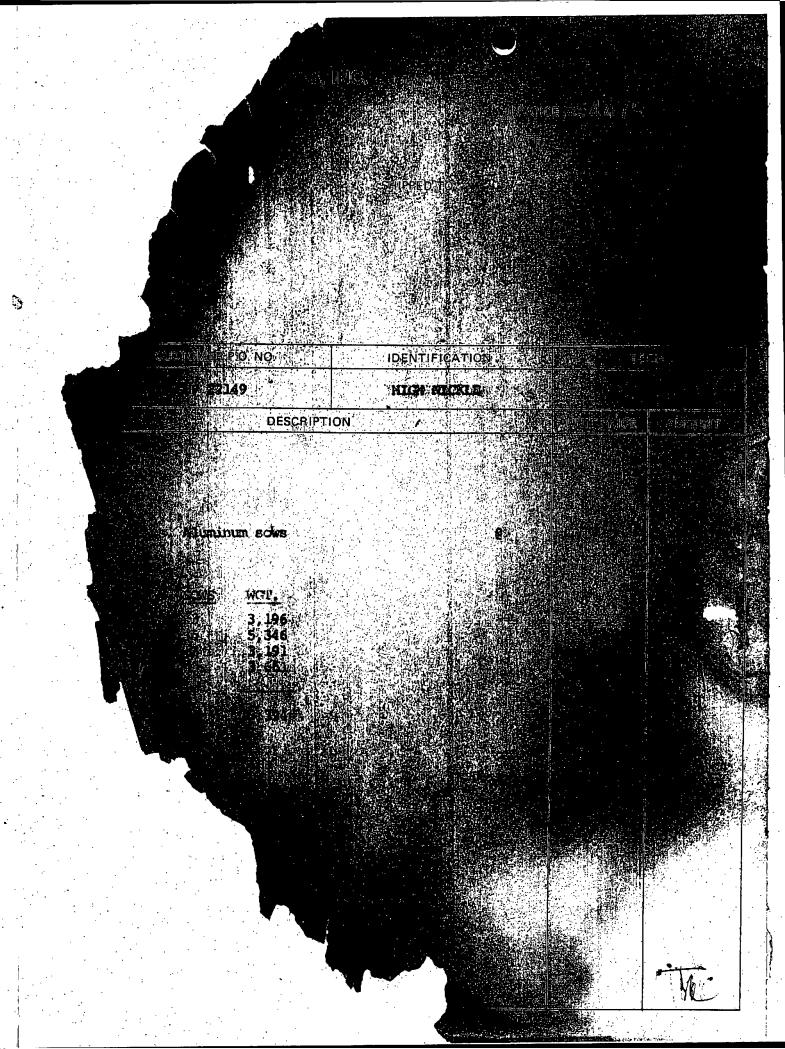




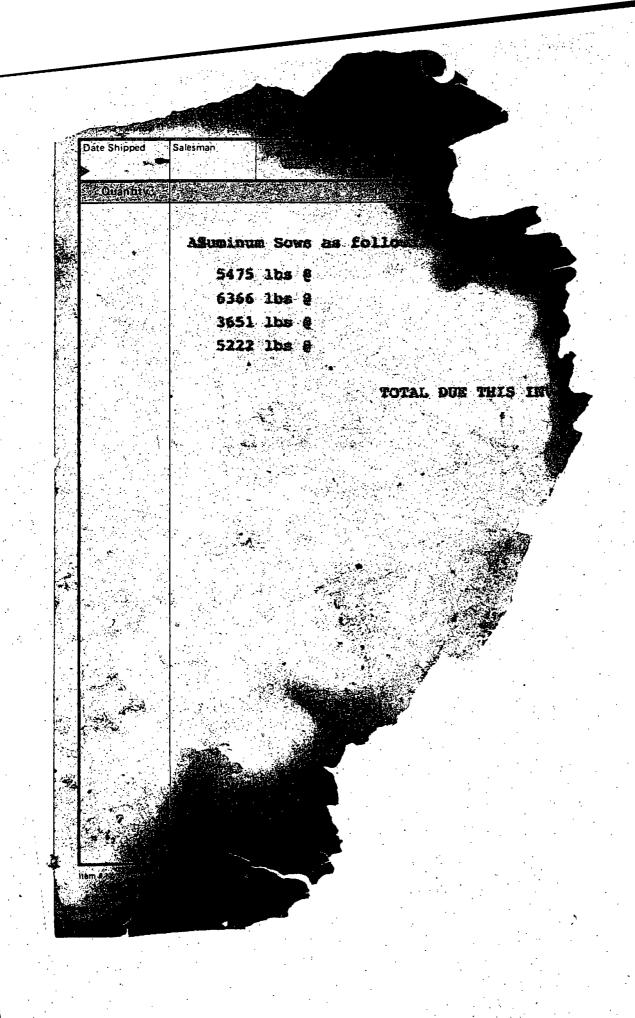


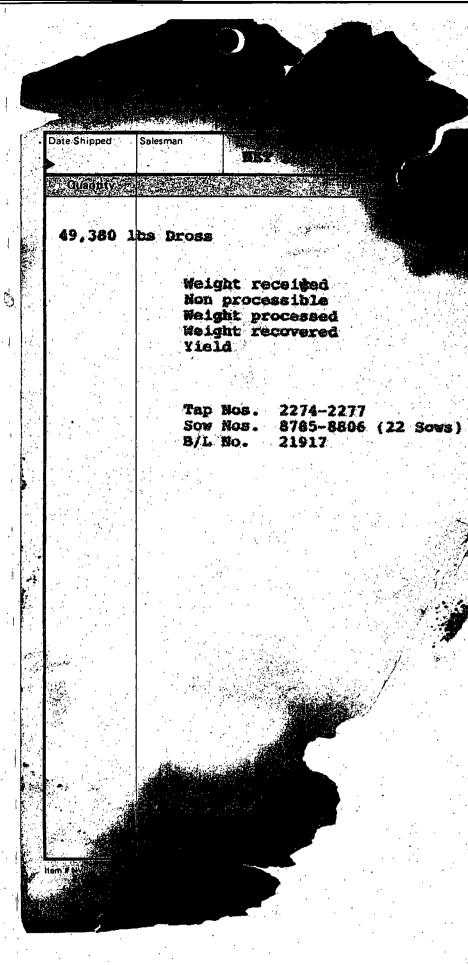


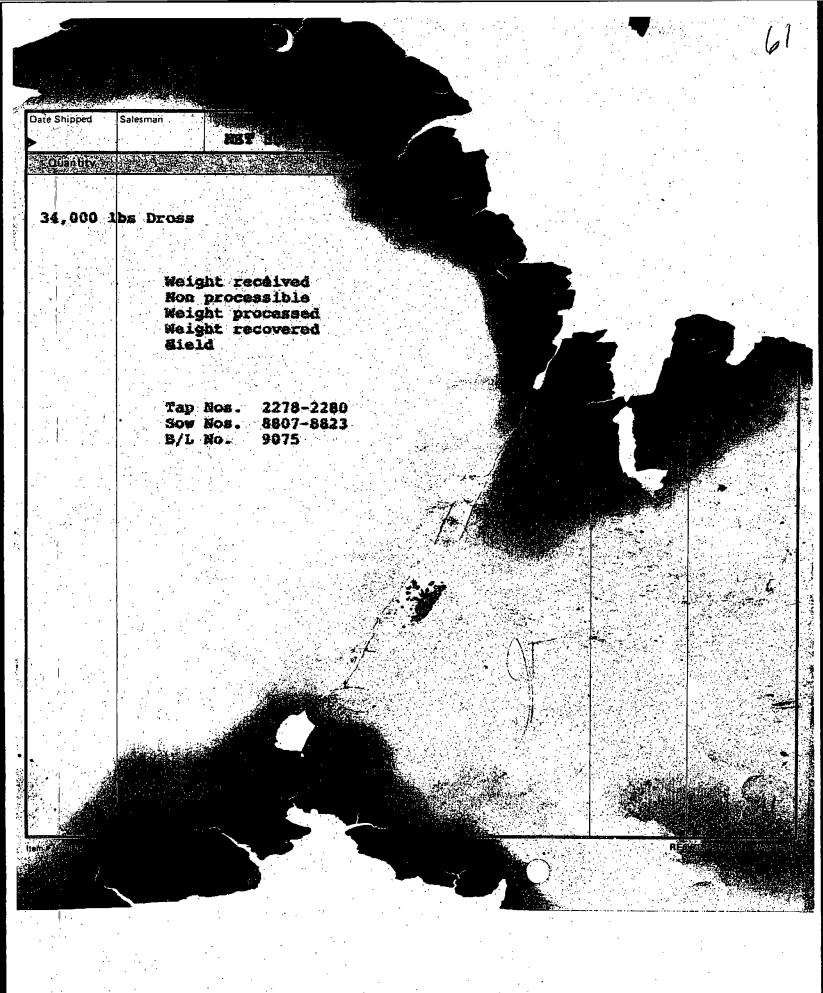


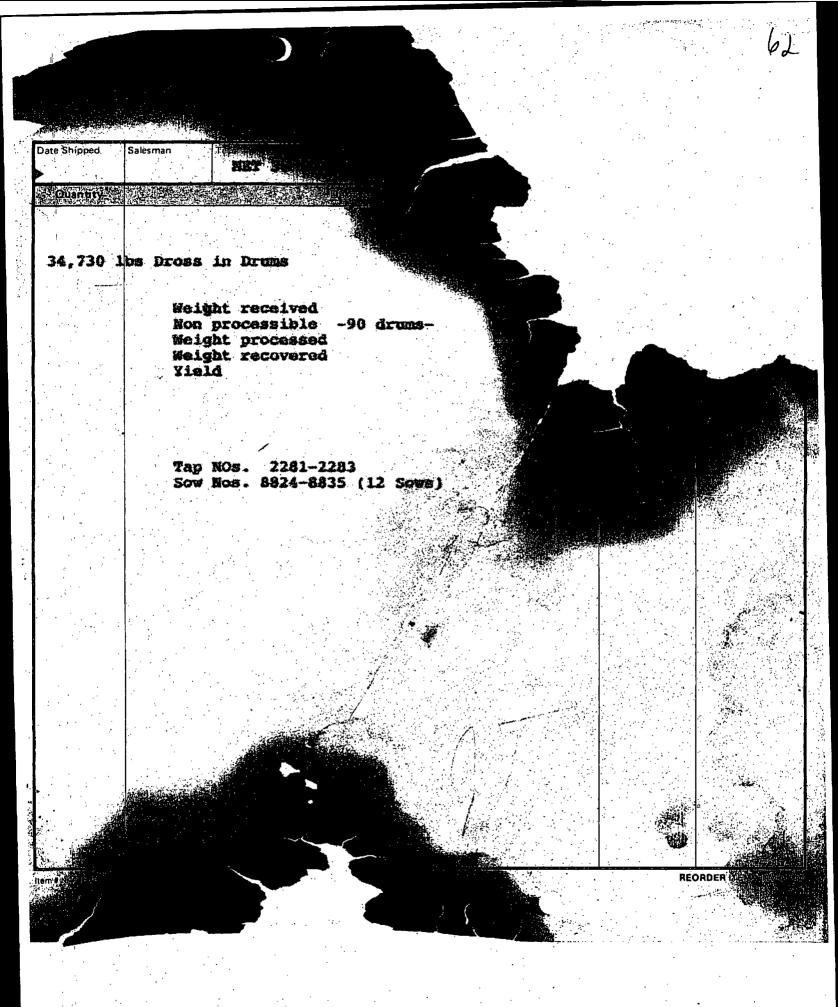


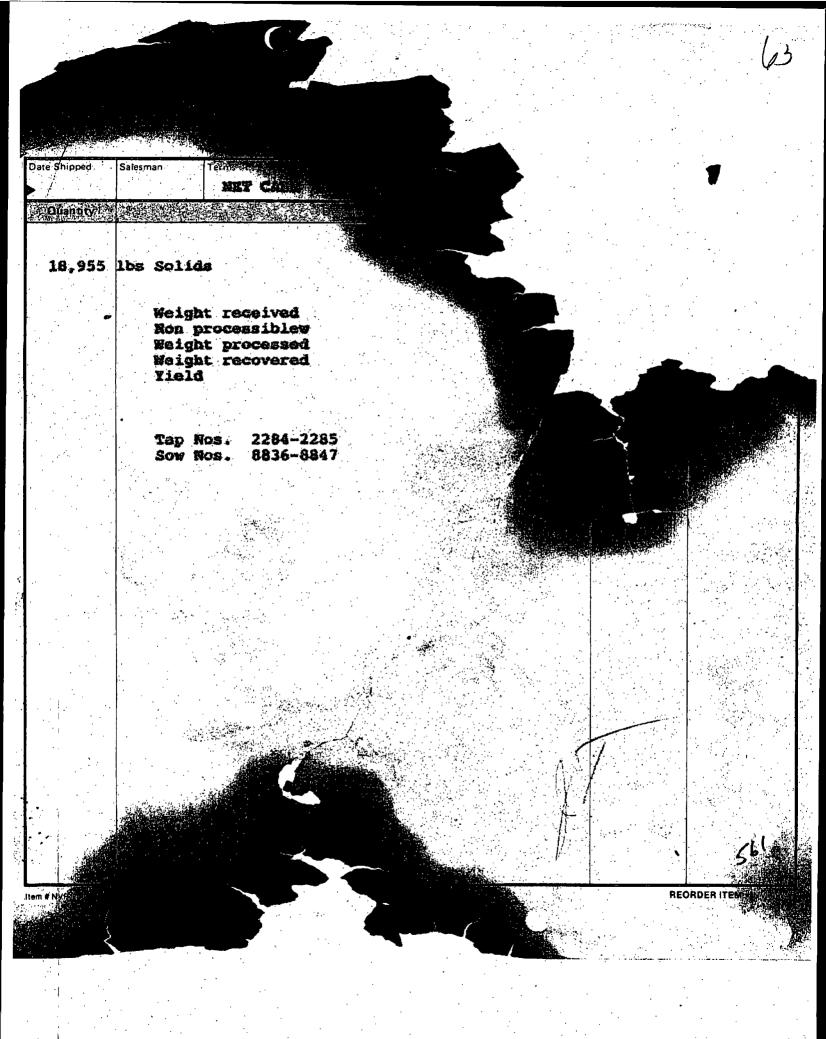


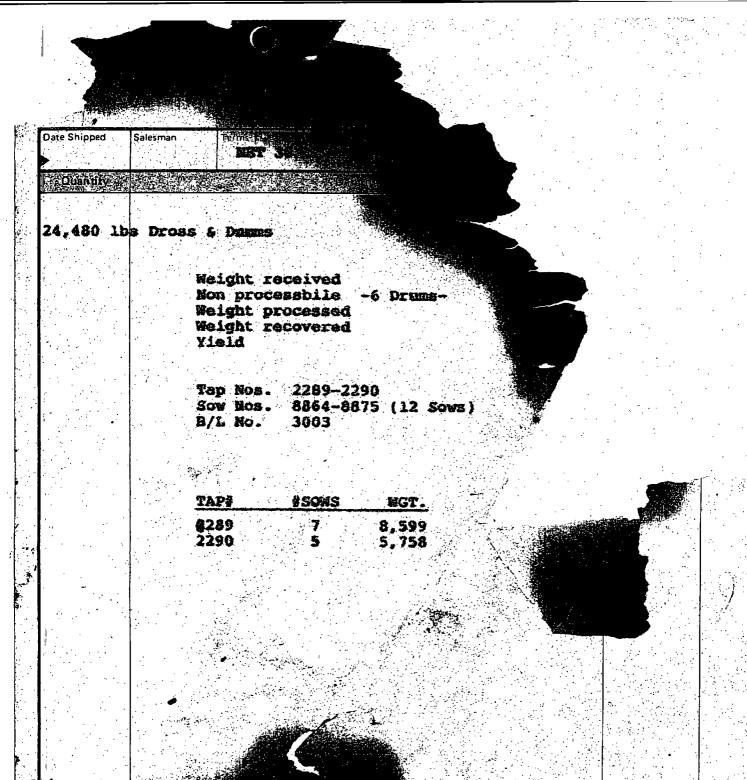






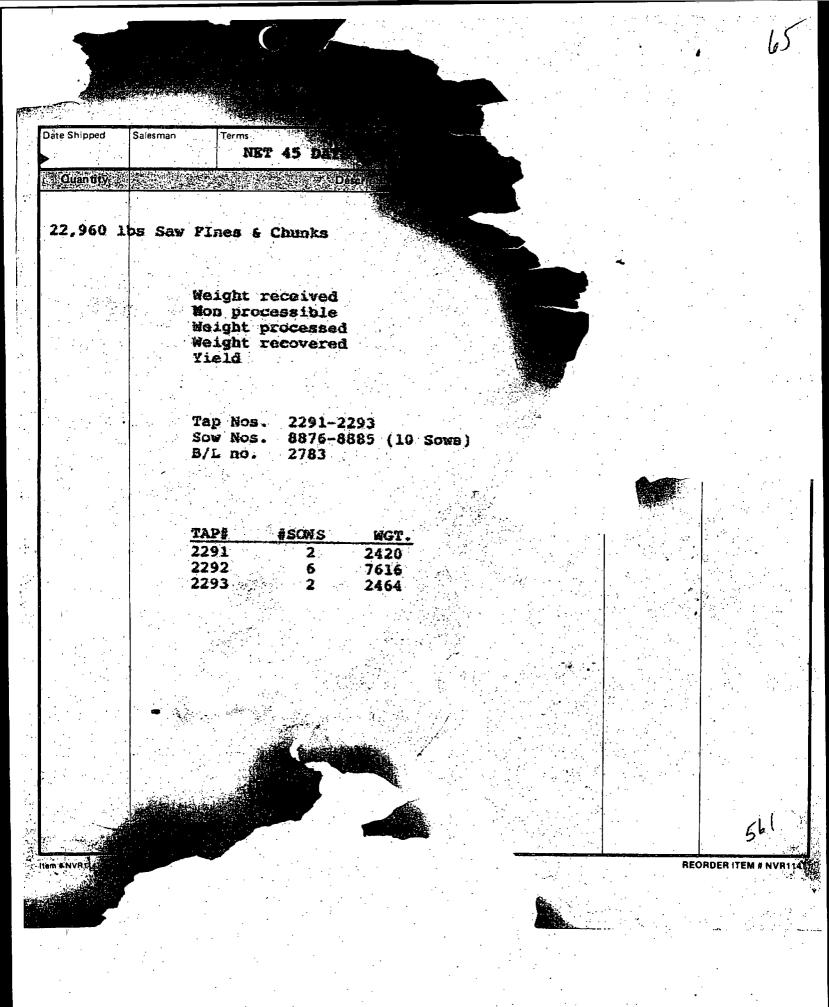


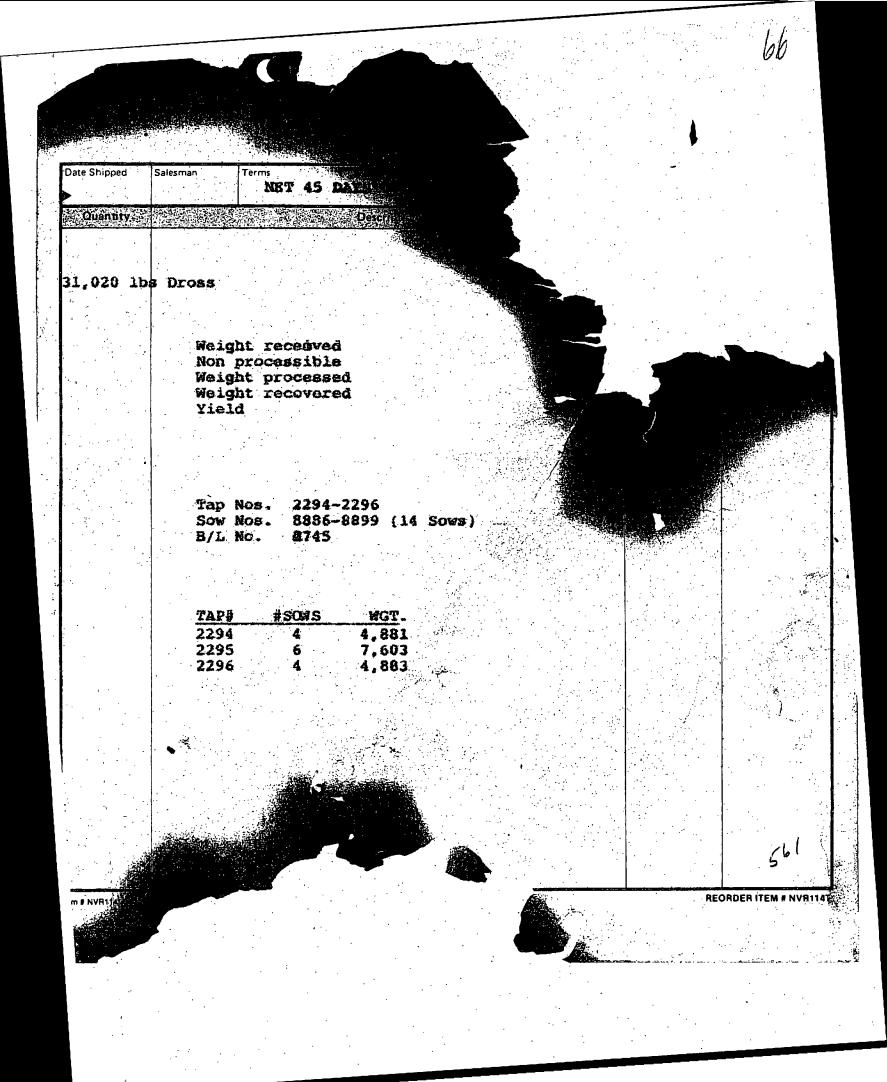


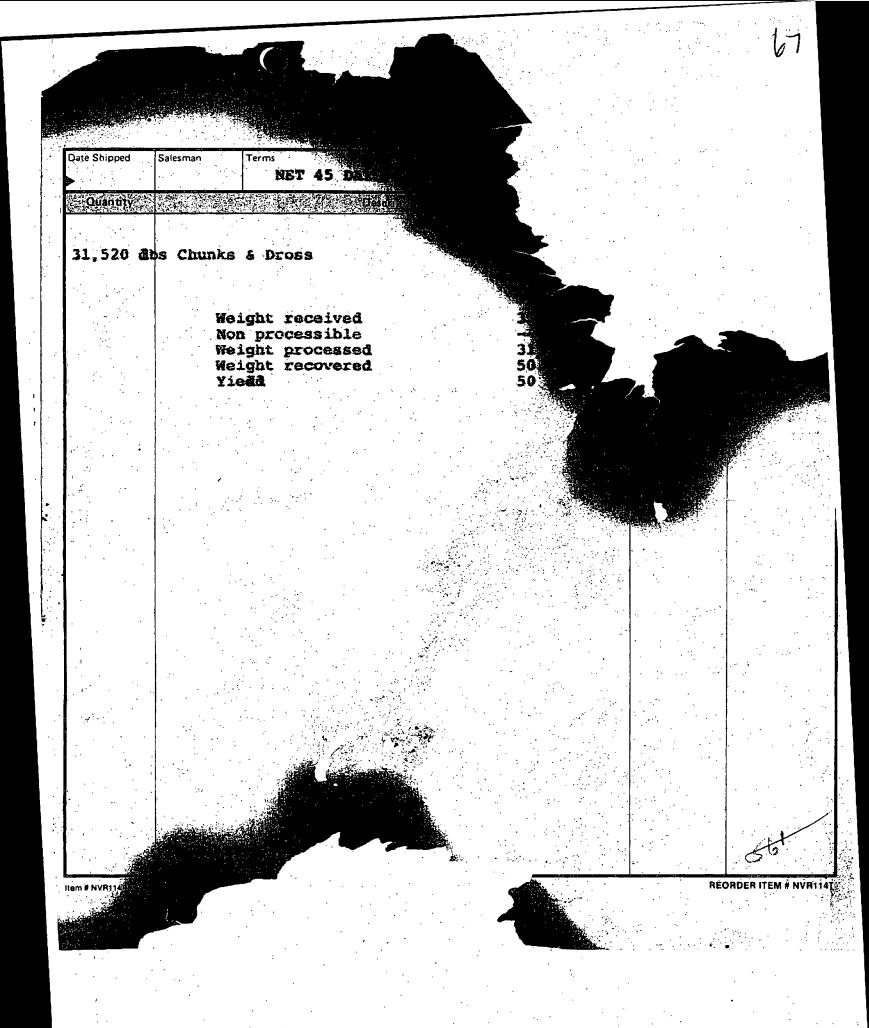


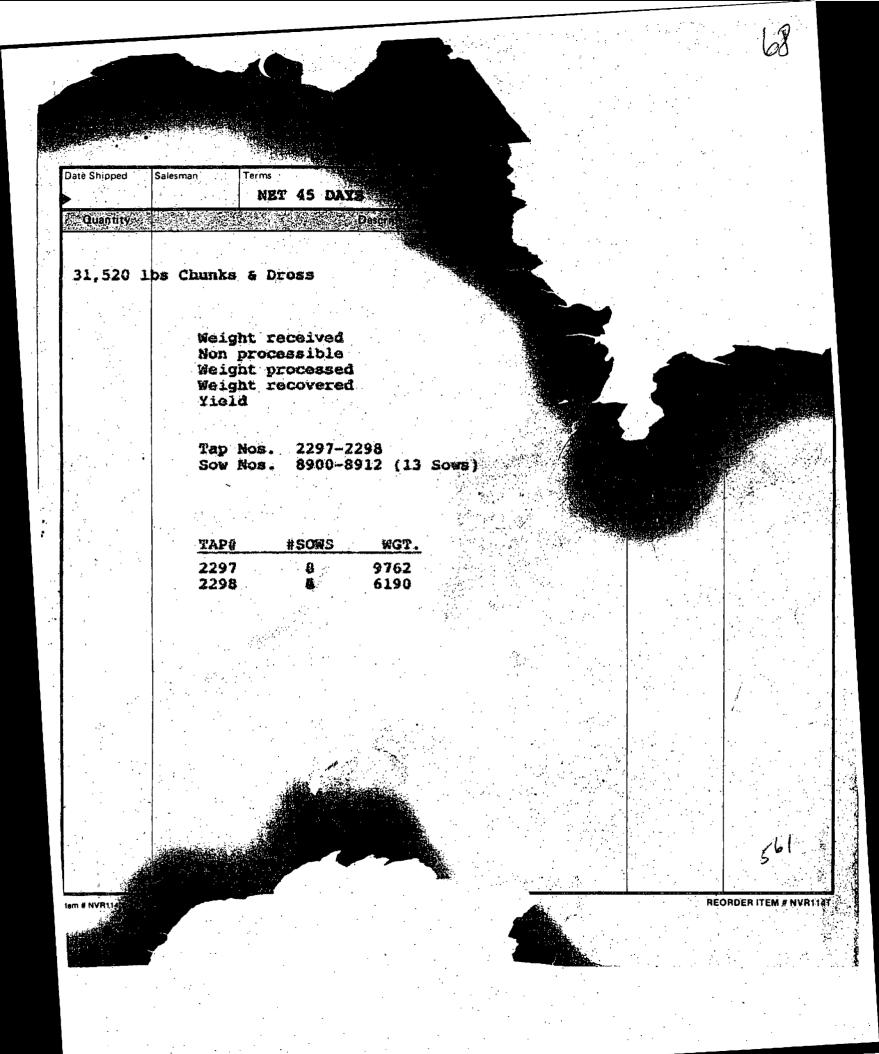
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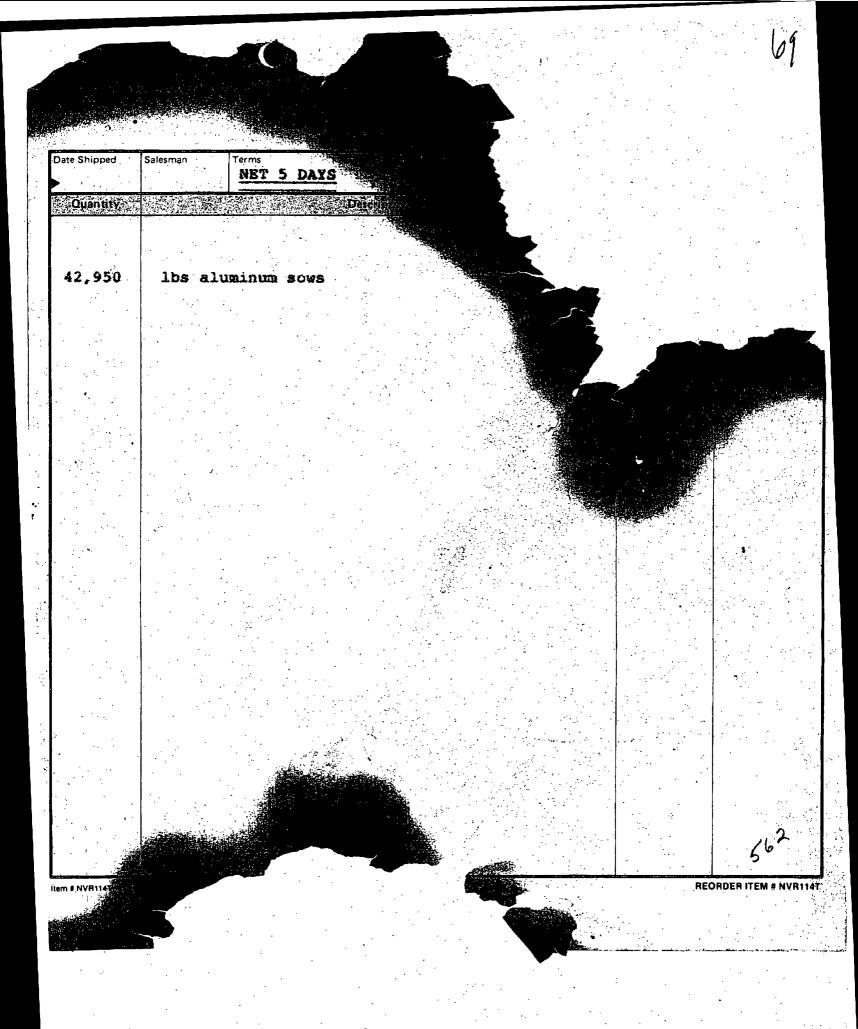
REORDER ITEM

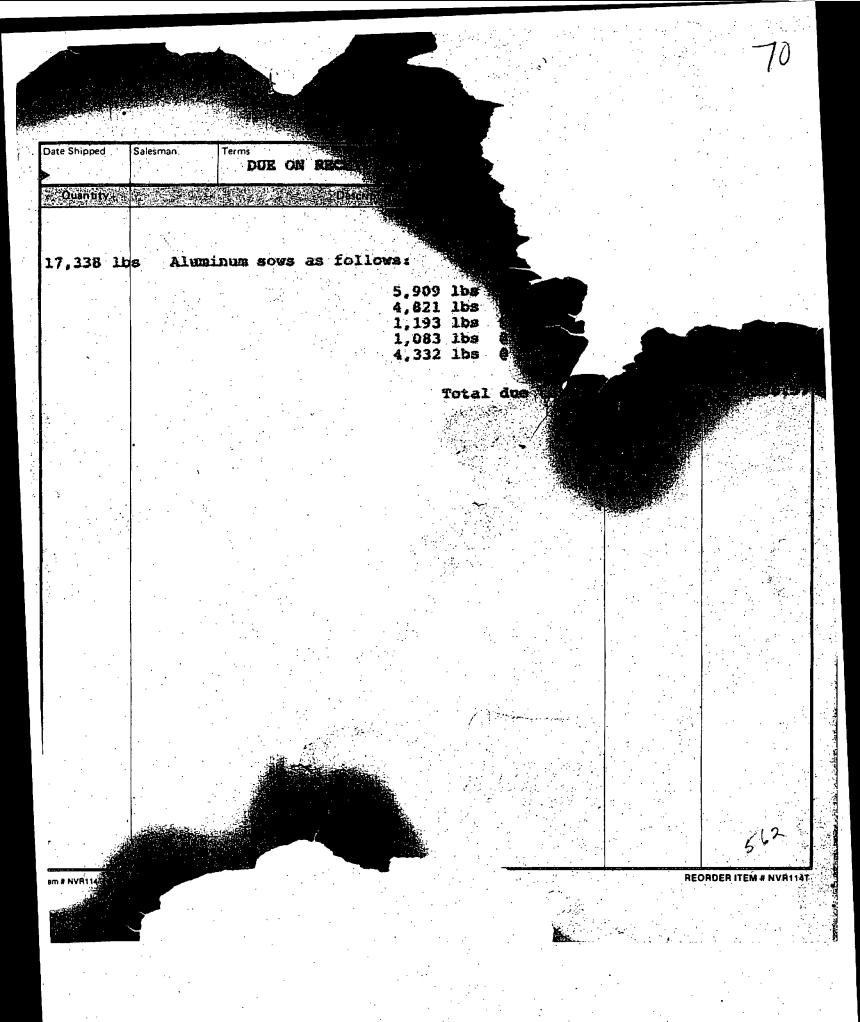












## Cover letter

Abstract approved by: Professor William J. Manning 10/7/2007

Potential reviewers:

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Aluminum accumulation in *Pteris cretica* and heavy metal uptake in vegetation growing on an abandoned aluminum smelter site in Knoxville, TN USA.

Jason M. Abercrombie<sup>a</sup>, Melanie Stewart<sup>b</sup>, Murali R. Rao<sup>a</sup>, Michael E. Essington<sup>b</sup>, and C. Neal Stewart, Jr. <sup>a</sup>

Corresponding author: C. Neal Stewart, Jr.

For correspondence: (phone: 865-974-6487; fax +1 865 946 1989; email

nealstewart@utk.edu)

Permanent address: Department of Plant Sciences, University of Tennessee, 2431

Joe Johnson Blvd., Knoxville, TN 37996-4561, USA

"Capsule": *Pteris cretica* accumulated aluminum instead of arsenic in the presence of high concentrations of aluminum found in smelter slag.

## **Abstract**

Smokey Mountain Smelters in Knoxville, Tennessee is an abandoned secondary aluminum smelter where smelter waste (slag) was dumped on site, potentially posing a threat to nearby human and ecosystem health. Metal concentrations in the slag (Al, As, Cd, Co, Cr, Cu, Ni, Pb, Se, Zn) were quantified by inductively-coupled plasma spectrophotometry (ICP) and characterized by x-ray diffraction (XRD). The highest metal concentrations observed were 223,000 mg kg<sup>-1</sup> Al, 281 mg kg<sup>-1</sup> As, 132 mg kg<sup>-1</sup> Se, and 2910 mg kg<sup>-1</sup> Cu. Metal uptake was quantified in leaves from plants growing on slag, as well as *Pteris cretica* plants employed to extract As from slag. Our data suggests that *P. cretica* accumulates Al in high concentrations, but not As, when grown in slag. Metal concentrations in vegetation growing on slag were lower than controls grown in

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uncontaminated soil, suggesting low metal availability in slag or exclusion mechanisms in roots.

*Keywords*: Phytoremediation, Heavy metals, Aluminum uptake, *Pteris cretica*, Hyperaccumulator

#### 1. Introduction

The smelting of heavy metals has resulted in extensive emissions of toxic metals into surrounding environmental media (Nriagu and Pacyna 1988). The environmental fate of emissions and waste generated during the smelting process is sometimes within close proximity to developed areas, thus posing a significant threat to human health. Smokey Mountain Smelters (SMS) is an abandoned secondary aluminum smelter in Knoxville, TN that was declared a Superfund site in 2001 due to massive piles of smelter slag and other unknown wastes over most of the ~12 hectare property. The site is located within a 1.6 km radius of numerous residential and commercial properties, wells, schools, churches, and directly adjacent to a large public housing complex (Figure 1). Because the slag material is exposed and uncovered, the surrounding community has no protection from pollution derived from groundwater leaching, stormwater runoff, and wind dispersal of the toxic metals.

Phytoremediation, or the use of plants to cleanup contaminated sites, may serve as an additional technology in the remediation of grossly polluted soils, such as the SMS site. Plants that can tolerate and thrive on metal-contaminated soils can prevent dispersal by creating a barrier to wind, but may also facilitate safer removal of contaminants via harvesting the above-ground shoot tissue, where metals may accumulate (Salt 1998). Surveys of native plants able to grow on metal-polluted land (e.g. slag spoils) provide more

useful candidates for creating a site-specific, vegetative cap because the plants are well-suited to local conditions (Remon et al. 2005). Vegetative caps may serve to prevent erosion of the barren and exposed areas, while at the same time, initiate the processes of soil formation and vegetative succession (Munshower 1993). Plants that have evolved mechanisms of metal tolerance generally employ one of two main strategies; (1) uptake and accumulation in the vacuole or cell wall or (2) exclusion via organic acid exudates and suppression of root transporters (Baker 1987).

Mounting evidence has demonstrated the utility of *Pteris vittata* for the phytoextraction of arsenic from contaminated soils (Ma et al. 2001; Wei et al 2006a; Wei et al. 2006b; Wei et al. 2007; Wang et al. 2006; Wang et al. 2007). This species is desirable for field-scale phytoextraction of arsenic due to its high biomass and accumulation of > 2% of its dry mass as arsenic (Wang et al. 2002). Fayiga et al. (2004) investigated the effects of heavy metals on the growth and arsenic accumulation in *Pteris vittata* in a greenhouse study. They found that the fern was able to hyperaccumulate arsenic despite the presence of Cd, Ni, Pb, and Zn. We decided to use *Pteris cretica*, an arsenic-hyperaccumulating relative of *Pteris vittata*, in this investigation. The presence of high As concentrations found in the slag material at the SMS site and the observation that wild vegetation could thrive on the slag piles warranted a test of the hypothesis that *Pteris cretica* could grow in the slag and extract As from the media. A preliminary greenhouse study using slag from the smelter site demonstrated that *Pteris cretica* grew at a reduced rate compared to controls, but showed no other signs of phytotoxicity (Supplementary Figure 1.), thus providing the impetus for a field-scale trial of its performance at the SMS site.

The SMS site offers a unique opportunity for scientific investigation due to the various plant species that grow without symptoms of toxicity and predominate on the otherwise barren landscape of the heavy metal-laden property. Therefore, the objectives of this study were to (1) characterize and quantify the trace metals found in the slag piles, (2) evaluate the shoot tissue for heavy metal accumulation in plants that were found thriving on the piles, and (3) evaluate the capacity of arsenic phytoextraction in *Pteris cretica* when challenged with the mixed slag waste.

## 2. Materials and Methods

Study site: site history, hydrogeologic setting, and EPA site inspection summary

The Smokey Mountain Smelter site is located in Knoxville, TN (83°55'36.77" West longitude and 35°55'06.68" North latitude). The ~12 hectare property is partially wooded, but significantly barren and covered with large piles of unknown wastes thought to derive primarily from the secondary aluminum processing facility that operated onsite from 1979 until the close of operations sometime after May 1994. Prior to Smokey Mountain Smelters and the existence of environmental regulations, the site was home to Knoxville Fertilizer Company from at least 1922 until 1948, and subsequently associated with several agricultural chemical manufacturing companies until 1965 (Maupin 2005). The agricultural facility could have discharged wastes into settling ponds (TDHE 1983). No information is known concerning the regulatory status of the site prior to 1980.

During the years of SMS operation, SMS received numerous citations from the local division of air pollution control due to numerous complaints from local residents (KCDAPC 1985; KCDAPC 1989). The site historically had a strong ammonia odor and

the waste was often burning (KCDAPC 1983). In addition to air pollution violations, the TN Division of Solid Waste Management issued a citation for operating a landfill without a permit and a geologic inspection of the site characterized the site as unsuitable for use as an industrial landfill (TDHE/DSWM 1983a; TDHE/DSWM 1983b). Materials that were incinerated in the smelters included by-products of primary aluminum production (i.e. aluminum dross, pot pads, pot bottoms, bath pads, and crushed material containing "nonprocessible" carbon, iron, cryolite (Na<sub>3</sub>AlF<sub>6</sub>), dust, etc. (Maupin 2005). Pot pads, pot bottoms, and bath pads are all generated inside of and in contact with spent potliners, which are listed hazardous wastes, designated as hazardous waste number K088 under the EPA Resource Conservation and Recovery Act (RCRA) (Maupin 2005). Maupin (2005) reported that between 1985 and 1992 SMS received large quantities of materials (oily scraper chips, furnace bottoms, magnetic separator accumulations, tabular balls, selee filters, south ingot furnace bottoms, mold line floor sweepings, can rec skim, and other miscellaneous materials derived from primary aluminum production) from a nearby primary Aluminum production facility in the city of Alcoa, TN. Large quantities of hazardous substances derived from the materials sent by the primary processing facility are known to still be present at the site (Maupin 2005).

The East Tennessee valley, in which the SMS site is situated, is oriented in a northeast-southwest direction as a result of folding and fracturing (TDC/DG 1956). The underlying geology of the SMS site is Middle Ordovician shale and characterized by extensive Karst development (TDC/DG 1956). Groundwater movement in such areas is restricted to largely interconnected fractures, thus potentially targeting approximately 2524 people that use groundwater in the 4 mi radius surrounding the SMS site (Maupin 2005).

Previous analytical results of groundwater samples from the site indicated the presence of antimony, arsenic, pentachlorophenol, dieldrin, and various toxic metals (except Cd), all exceeding the primary drinking water maximum contaminant levels as declared by the US EPA (Maupin 2005). This report also concluded that the onsite, unlined waste lagoon posed a serious threat to groundwater quality, considering the permeable subsurface. Similar conclusions were also reported for the vulnerability of local wetlands and downstream fisheries.

As a result of the aforementioned evidence, the TN Division of Remediation site inspection report suggested that the SMS site has potential to be placed on the National Priorities List for cleanup and recommended immediate remedial action. It is clear that trespassing occurs on the site by local children (Supplementary Figure 2); the adjacent housing project, Montgomery Village, currently houses hundreds of individuals and an elementary school is located approximately 1.6 kilometers away. For example, large gaps in the chain-link fencing on the side facing the adjacent public housing complex, well worn footpaths leading between the complex, and the predominance of graffiti within the dilapidated warehouse surrounding the rotary furnaces of the smelter (Supplementary Figure 2) raise obvious concerns for the health of local children.

# Soil and plant sampling

During the month of May 2006, leaves were sampled from plants growing on smelter waste piles, but displaying no apparent symptoms of toxicity. Species selected for analysis were those found in multiple locations among the slag heaps, initially leading us to speculate that these species were employing some mechanisms of tolerance to high

concentrations of several heavy metals. These species were: *Verbascum thapsus*, *Lirodendron tulipifera, Carduus nutans, Solidago canadensis, Ailanthus altissima*, *Parthenocissus quinquefolia, Platanus occidentalis*, and *Phytolacca americana*.

Representative control samples for each species collected at the site were taken from an uncontaminated area adjacent to the Superfund site. Leaf samples were only collected for each species that appeared to best represent a mirror image of plant age, which was performed by on-site comparisons of leaf size and plant height. Each treatment (species) sampled consisted of ≥4 biological replicates.

Soil samples were taken with an auger to represent a depth ranging from 0-20 cm. All soil samples were air dried and sieved to < 2 mm prior to analysis. Each soil sampling was performed in triplicate at each plot from the *Pteris cretica* experiment, as well as the uncontaminated control plots. Due to the high clay content of the soil found in the nearby representative control soil samples (A, B, C; Figure 1), less compact soil with a higher organic content was selected for the *Pteris cretica* planting controls.

## Pteris cretica experiment

Six 1 m<sup>2</sup> plots of waste soil were chosen to span the spatial range of the site for uptake experiments using *Pteris cretica* cv. Mayii. Within each plot, fifteen 5-month-old ferns were transplanted, spaced 20 cm apart, fertilized with 20-20-20 N:P:K Osmocote® fertilizer, and treated with a 3 kg application of lime. Shade cloth structures were employed randomly to three of the six plots, as well as one of the two control plots which were located adjacent to the contaminated area on uncontaminated soil. All plots were watered as needed with water from the on-site waste lagoon. After two months of growth in

the field (June 1 – July  $30^{th}$ , 2006), above-ground biomass for each sample was harvested for metal analysis.

Sample preparation and chemical analysis

Soil, smelter slag, and plant tissue total metals (Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se, Zn) were determined by a SPECTRO CIROS CCD EOP inductively coupled plasma spectrophotometer (ICP) with an AS400 autosampler (SPECTRO Analytical Instruments, Kleve, Germany). Three and a half grams were subjected to a 4 M nitric acid overnight reflux at 70 °C according to Chang et al. (1984). One gram of leaf tissue from each plant analyzed was oven-dried at 60 °C for 72 hrs and ashed in a muffle furnace at 450 °C overnight. Ashed samples were digested with 10 ml of 1 N HNO<sub>3</sub>, heated to dryness, then warmed to near boiling in 10 ml of HCl. Samples were brought to 50 ml volume with DDI H<sub>2</sub>0 and filtered using Whatman no. 42 paper prior to ICP analysis. To further characterize the mineralogy of the smelter slag, samples were subjected to x-ray diffraction (XRD) analysis using a D8 Advance with a K760 generator (Bruker AXS, Inc., Madison, WI). A simple fizz test was performed by adding a few drops of 10% HCl to confirm the presence of calcium carbonate in the slag material. Soil and slag pH was determined after a 1 hr shaking incubation of a 1:1 mixture of soil: 0.1 M CaCl<sub>2</sub>.

## Statistical analysis

Because all field data did not meet the assumptions for normality, a one-way Mann-Whitney U test was employed to evaluate the differences in mean metal uptake between

slag-grown and control wild vegetation, as well as in comparisons of mean slag and control soil metal concentrations using JMP statistical software (SAS Inc., Cary, NC).

### 3. Results

3.1. Soil pH and heavy metal content of smelter slag and adjacent control soils

Table 1 summarizes the heavy metal content (Al, As, Cu, Cr, Cd, Co, Se, Ni, Pb, Zn) and pH of each designated plot (1-6) located within the smelter slag waste area, as well as the nearby uncontaminated soils (A-D), the locations of which are all indicated in Figure 1. Pb was not detected in any of the slag samples, but was present at low concentrations (>7 mg kg<sup>-1</sup>) in control soils A-C (data not shown). XRD analysis of the slag material revealed the presence of polymorphs bayerite and gibbsite [Al(OH)3], spinel (MgAl<sub>2</sub>O<sub>4</sub>), calcite or CaCO<sub>3</sub>, and calcium aluminum oxide (Ca<sub>3</sub>Al<sub>2</sub>O<sub>6</sub>) (Supplemental Figure 3). Statistical comparisons of the mean metal content in slag and control soils reveal significant differences between slag material and control soils (Table 1). No significant differences in metal content were found to exist between the two depths assayed. Slag raw pH values ranged from 7.33 to 8.27, whereas raw pH values for the control soils ranged from 3.86 to 7.66 (Table 1). Metal content and pH for uncontaminated control soil employed in the Pteris cretica experiment is represented by control soil D (Table 1). Although relatively high in Al, As was not detected by ICP analysis of this soil. Additionally, Zn, Ni, Cu, Cr, and Co, were all relatively low in the fern control soil.

#### 3.3 Heavy metal uptake in slag-grown Pteris cretica

Figure 2 illustrates the metal content in fronds of field-grown *P. cretica* plants harvested two months after planting. Despite the absence of As in the control soils used for this experiment, As uptake was observed to be restricted in the slag-grown ferns (Figure 2). However, Al was accumulated in high concentrations in the slag-grown ferns, ranging from 569 mg kg<sup>-1</sup> to 4 380 mg kg<sup>-1</sup>, and averaging 1 821 mg kg<sup>-1</sup>. Slag-grown ferns also accumulated significantly higher amounts of Cu, Mo, Se, Zn, and Ni than were observed for control ferns.

## 3.2. Heavy metal uptake in slag-grown vegetation and bioaccumulation factors

A comparison of the heavy metal content in leaves of wild vegetation found growing on the slag heaps versus nearby uncontaminated control soils is illustrated in Figure 3. Surprisingly, restricted uptake of several species analyzed was indicated by significantly higher metal uptake in plants harvested offsite on uncontaminated soil compared to the slag-grown plants (Figure 3.), especially evident for the uptake of As, Se, Cu, and Co. One exception to this trend was demonstrated by the pioneering species *Verbascum thapsus*, accumulating the highest levels of aluminum uptake among slaggrown plants, as well as among controls (323 mg kg<sup>-1</sup> and 137 mg kg<sup>-1</sup>, respectively). Slaggrown *V. thapsus* also exhibited the highest Ni and As uptake, however all control plants exhibited higher As uptake compared to the slag-grown plants. *Carduus nutans* growing offsite accumulated the highest levels of As, Cr, Co, and Cd. *Phytolacca americana* contained the highest concentrations of Zn in leaf tissues harvested from plants growing in uncontaminated soil.

Bioconcentration factors (BCFs) (i.e. the ratio of metal concentrations in the shoot to those in the soil) indicate the efficiency at which plants extract metals from the soil. Slag-grown *P. cretica* exhibited the highest BCF values with respect to Al and Se (Figure 4). All plant species analyzed from uncontaminated soil had significantly higher BCFs for Al, As, Cu, Cr, Se, Ni, and Cd, with the exception of slag-grown *P. cretica* which exhibited much higher BCFs for Al, As, and Se. BCF for Zn in *P. cretica* grown in uncontaminated soil exceeded 50, dramatically higher than any other species. *Carduus nutans* grown in uncontaminated soil had the highest BCFs for As, Ni, and Cd.

#### 4. Discussion

## 4.1 Heavy metal uptake and bioaccumulation factors in wild vegetation

Numerous investigators have surveyed wild plant populations growing on or nearby heavy metal-contaminated sites, generally with intentions of discovering species with novel remediation traits, such as heavy metal tolerance and accumulation. Del Rio et al. (2002) studied trace metal uptake in 99 wild plant species growing in an area contaminated from a spill of toxic pyretic sludge. These authors identified 11 plant species that demonstrated promising utility in the phytoremediation of Pb, Zn, Cu, Cd, and As due to their observed metal tolerance, accumulation, and high biomass. If phytoextraction is not a feasible means of remediation, then natural revegetation of a contaminated site can serve to stabilize the contamination by minimizing stormwater runoff and wind dispersal (Vangronsveld et al. 1995). In the current study, most plants exhibited significantly lower concentrations of trace elements in their leaves compared to control plants (Figure 2), thereby suggesting that

either these plants were able to survive in the metalliferous medium via exclusion mechanisms or due to the neutral pH range of the slag and the abundance of aluminum (hydro)oxides, trace metal adsorption resulted in very low metal availability. Similar reports by Gonzalez and Gonzalez-Chavez (2006) suggest that most plants growing near mining wastes were employing exclusion mechanisms because metals did not accumulate in shoot tissues despite the high concentrations found in the soil. Despite the exclusion behavior found in those plants, two species, *Polygonum aviculare* and *Jatropha dioica* were reported to accumulate Zn at concentrations near the criteria for hyperaccumulation. Similarly, we report that slag-grown *Verbascum thapsus* was not only tolerant to the slag metal concentrations, but exhibited significantly higher accumulation of Ni and Al than those growing in uncontaminated soil (Figure 2). Regardless, the highest metal content observed in V. thapsus (462 mg kg<sup>-1</sup>) was lower than sufficient for hyperaccumulator status. In a recent study of trace element (Cu, Fe, Mn, Ni, Pb, Zn) content in a close relative, Verbascum olympicum, Guleryuz et al. (2006) reported that heavy metal contents in different organs were highly correlated to heavy metal content found in the soil.

The BCF values for these species demonstrate that most of the wild species have no reasonable utility for employment in phytoextraction at the SMS site. McGrath and Zhao (2003) demonstrate that the two key components necessary for a plant to be of feasible utility in the phytoextraction of heavy metals are high biomass and high BCF (i.e. the metal concentrations in the above-ground biomass should exceed those found in the soil). Some of the species that grew on the slag exhibited substantial biomass (Supplementary Figure S2h). One obvious explanation for the low BCF values observed for slag-grown plants is the remarkably high metal concentrations found in the slag (Table 1). Even in the control

plants that exhibited significantly higher BCFs than slag-grown plants, no wild species had a BCF close to 1. McGrath and Zhao (2003) present a useful model for selecting feasible candidates for phytoextraction. According to these authors, in order to reduce the metal concentration in the top 20 cm of topsoil by half, with a BCF of 1, even high biomass crops (20 t ha<sup>-1</sup>) would require approximately 100 crop harvests.

## 4.2 Trace element uptake in Pteris cretica

Arsenic hyperaccumulation in members of the *Pteris* genus is well documented and the list of field-capable As-hyperaccumlating *Pteris* species and cultivars continues to grow as surveys of *Pteris* taxa found at As-contaminated sites are being conducted (Wei et al 2006; Wei et al. 2007; Wang et al. 2006; Wang et al. 2007). We selected *P. cretica* cv. Mayii because of its proven field-scale and hydroponic performance in As hyperaccumulation (Wei et al. 2006; Poynton et al. 2004; Fayiga et al. 2005). We were primarily interested in whether the fern was capable of extracting the high concentrations of As found in the slag material. Interestingly, the fern accumulated high amounts of Al during the 8 weeks of growth in the slag waste.

The most common criteria for a plant to be considered a hyperaccumulator is that the shoot metal concentration must exceed 1.0% for Zn and Mn, 0.1% for Al, As, Se, Ni, Co, Cr, Cu, and Pb and 0.01% for Cd (Branquinho et al. 2007). In this study, *P. cretica* clearly met these criteria for Al, however the extremely high concentrations of Al found in the growth medium (slag) contributed to a low BCF value, thus negating a characterization of *P. cretica* as a hyperaccumulator of aluminum at this time. However, the interesting observation that *P. cretica* exhibited a BCF of over 50 for Zn uptake (Figure 4) warrants

further investigation into the uptake dynamics of *P. cretica* under Zn-deficient conditions, as was observed in control soils used in the *P. cretica* experiment. These results are similar to those found by An et al. (2006) who reported Zn tolerance and accumulation in *Pteris vittata*. These authors demonstrated that *P. vittata* accumulated up to 737 mg kg<sup>-1</sup> Zn in fronds in the field, but could also accumulate As under high Zn concentrations, suggesting that *P. vittata* could be useful in sites co-contaminated with Zn and As. We did not detect the presence of As in the uncontaminated soil used for the *P. cretica* control plants in this study, thus explaining the very low amount of As observed in these plants.

Although aluminum (hydro)oxides were the predominant minerals found in the SMS slag, these minerals are also ubiquitous in soils and affect the fate and transport of ionic pollutants (Cox and Ghosh, 1994). Cox and Ghosh (1994) demonstrated that the adsorption of As(V), CH<sub>3</sub>AsO(OH)<sub>2</sub>, and (CH<sub>3</sub>)<sub>2</sub>AsOOH to amorphous Al(OH)<sub>3</sub>, gibbsite, α-Al<sub>2</sub>O<sub>3</sub>, and γ-AlO<sub>3</sub> increased up to pH 7, but decreased sharply at higher pH values. Extractions to determine the bioavailability of As in the slag material were not performed. However, based upon the uptake measurements observed in slag-grown *P. cretica*, we believe that the arsenic is likely adsorbed to the aluminum oxides/hydroxides found to predominate in the slag, thereby making As unavailable for uptake. This phenomenon has recently been reported in a study that characterized arsenate adsorption on aluminum oxide and phyllosilicate mineral surfaces in smelter-impacted soils (Beaulieu et al. 2005). These authors suggested that As originally released from the smelter was oxidized, dissolved, and adsorbed onto soil minerals and that the mildly acidic pH conditions found in the soil allowed for stable sorption complexes, thus preventing significant As mobilization. It is

known that As mobility in soils is affected by pH (Adriano 1986). A recent study of As mobility in sites impacted by As mining and smelting suggested that As is mobile at extreme pH values (<2 or >8), such as those observed in mine tailings and tailings-impacted alluvial soils, however all other soils exhibited very low As mobility (Krysiak and Karczewska, 2007). Our data only reflect the pH values found in the top 20 cm of the slag piles, however since some of the plots exhibited pH values near 8 and greater (Table 1; plot 4 and 5), pH and resulting absorptive capacities in deeper zones may differ from those at the slag surface, thereby potentially mobilizing Al-bound As species into the groundwater.

## 4.3 Aluminum tolerance and accumulation

Aluminum toxicity in acid soils is a global agricultural dilemna, therefore much attention has been given towards understanding Al tolerance in plants. Attempts to identify genes involved in Al tolerance have been made in studies of Arabidopsis, wheat, barley, and, rye (reviewed by Magalhaes 2006). A major mechanism of Al tolerance that has been elucidated in these species is associated with the chelation of Al via exudation of the organic acid malate from the root apex, thereby preventing Al uptake via exclusion (Magalhaes 2006).

Conversely, Al accumulation has been reported for 127 species within the Melastomataceae family in the Order Myrtales, however the mechanisms through which this occurs are unknown (Jansen et al. 2002). Al accumulators have also been reported for members of the Rubiaceae and it is believed that these plants may utilize Si for Al detoxification, as relatively high Si levels were also observed in these plants (Jansen et al.

2003). However, this finding has not been confirmed because the Si: Al mole ratio widely varied among species (Jansen et al. 2003). The Al uptake in *Pteris cretica* observed in this study certainly warrants follow-up investigation. Because of the high number of environmental variables found in a field-scale study such as this, more controlled experiments are needed, paying particular attention to effects of pH and nutrient availability on Al content in various tissues. However, high Al accumulation was observed for all six plots, spanning a wide spatial range at the study site, thereby suggesting that the ferns are able to employ some mechanisms of accumulation.

Our data suggest that for wild vegetation growing on slag, Al is excluded, possibly via exudation of organic acids (i.e. malate, citrate) similar to well-characterized Al tolerance mechanisms. It is known that the solubility of Al is significantly reduced at pH 5.0 and above (Reid et al. 1971). Additionally, elevated concentrations of cations (i.e.  $Ca^{2+}$ ,  $Mg^{2+}$ ) in the rhizosphere are known to ameliorate Al toxicity (Brady et al 1993; Kinraide et al 1992). XRD analyses have revealed the presence of spinel (MgAl<sub>2</sub>O<sub>4</sub>), calcite or CaCO<sub>3</sub>, and calcium aluminum oxide (Ca<sub>3</sub>Al<sub>2</sub>O<sub>6</sub>), indicating the presence of such cations, thereby leading us to speculate that despite the abundantly high concentrations of Al found in the slag, the effects of pH and abundance of  $Ca^{2+}$  and  $Mg^{2+}$  ions likely decrease the concentration of Al<sup>3+</sup> ions and ameliorate the phytotoxicity of the slag Al.

#### 5. Conclusions

Our discovery that aluminum accumulation occurs in *Pteris cretica* when challenged with slag containing high concentrations of other toxic metals and metalloids

has prompted us to investigate this phenomenon under controlled experimental conditions. We are particularly interested in the dynamics of As and Al uptake in *P. cretica* in the context of their bioavailability. Also as a result of this study, we intend to characterize the capacity for *P. cretica* to accumulate Cu and Zn.

It would be unreasonable to employ phytoremediation to cleanup a site as grossly contaminated as the SMS site, however this study has provided new insights that extend beyond phytoextraction. The windborne dispersal of metals at the SMS site may be decreased by continued succession of the known tolerant ecotypes found growing directly on the slag material. Therefore, phytostabilization of the slag may serve as an interim measure in prevention of contaminant spread that precedes appropriate remediation of this potential danger to the local community.

Additionally, we have identified several plant species as candidates for further study that may contribute to our understanding of aluminum tolerance. Because Al toxicity is such a significant global agricultural problem, exploring the molecular mechanisms that play a role in the exclusion of Al in the species evaluated in this study is warranted.

#### Acknowledgements

This work was supported by a grant from the National Science Foundation (OII-0548751). We are very grateful to Burl Maupin and the Knoxville Division of Remediation, Renee Hoyes at the Tennessee Clean Water Network (Knoxville, TN), and Barry Salkin at Public Employees for Environmental Responsibility (Nashville, TN) for facilitating this study. We appreciate our collaborators at Edenspace Systems Corporation for their

valuable cooperation, especially, Mark Elless, David Lee, and Bruce Ferguson. The authors are grateful to Dwayne Estes of the University of Tennessee herbarium for assistance with botanical determinations. We appreciate the help of Hong-Seok Moon and Brian Leckie for collection of slag to enable preliminary experiments.

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## Figure legends

- Figure 1. Satellite image of the abandoned Smokey Mountain Smelter site in South Knoxville, TN, USA (Image from Google<sup>TM</sup> Earth). The large building on the property houses two rotary furnaces and an incinerator. Slag from the smelting process now exists in piles over much of the property. Numbers 1-6 and respective black spots designate plots of *Pteris cretica* plantings and slag sampling. The white line outlines the waste lagoon. Letters A-D indicate approximate locations of uncontaminated soil sampling. The right side of the image shows the adjacent public housing project.
- Figure 2. Trace element accumulation (mg kg<sup>-1</sup>) in *Pteris cretica* grown on slag piles at the SMS site for 8 weeks. Black bars indicate plants grown on slag piles and grey bars refer to plants grown in uncontaminated control soil D (Table 1).
- Figure 3. Trace element accumulation (mg kg<sup>-1</sup>) in wild vegetation found growing on slag piles at the SMS site. Species represented are *Verbascum thapsus*, *Lirodendron tulipifera*, *Carduus nutans*, *Solidago canadensis*, *Ailanthus altissima*, *Parthenocissus quinquefolia*, *Platanus occidentalis*, and *Phytolacca americana*. Black bars indicate plants grown on slag

piles and grey bars refer to plants grown in uncontaminated control soils represented by controls A, B, and C (Table 1).

Figure 4. Bioconcentration factors (BCF) of trace elements in slag-grown wild vegetation and *Pteris cretica*. Species represented are *Verbascum thapsus*, *Lirodendron tulipifera*, *Carduus nutans*, *Solidago canadensis*, *Ailanthus altissima*, *Parthenocissus quinquefolia*, *Platanus occidentalis*, *Phytolacca americana* and *Pteris cretica*. Black bars indicate plants grown on slag piles and grey bars refer to plants grown in uncontaminated control soils. Control BCFs for wild vegetation were calculated from control soils A, B, and C, whereas BCFs for *Pteris cretica* controls were derived from control soil D.

**Tables** 

Table 1 Heavy metal concentrations (mg kg<sup>-1</sup>) in smelter slag (plots 1-6) and uncontaminated control soil (A-C) expressed as mean  $\pm$  sd. P values represent one-way Mann-Whitney U test comparisons of slag and control means.

plot	depth (cm)	рН	Al	As	Zn	Se	Ni	Cu	Cr	Co	Cd
1	1-10	7.65	178 186.1 ± 12 944.9	196.1 ± 14.0	318.4 ± 128.8	19.7 ± 1.2	333.3 ± 105.5	924.8 ± 378.4	47.8 ± 4.4	$6.6 \pm 0.8$	15.2 ± 1.0
	10-20	7.60	169 653.2 ± 19 257.2	182.3 ± 20.0	457.2 ± 196.8	21.8 ± 4.2	239.0 ± 118.5	790.8 ± 398.4	49.2 ± 11.8	$5.8 \pm 0.3$	14.2 ± 1.4
2	1-10	7.64	203 842.6 ± 5 872.1	219.6 ± 5.7	610.8 ± 298.2	$25.2 \pm 6.8$	1 196.5 ± 648.7	999.0 ± 775.0	67.6 ± 23.0	$7.9 \pm 2.9$	$17.0 \pm 0.7$
	10-20	7.58	189 689.3 ± 29 481.8	204.0 ± 27.3	233.4 ± 118.0	17.9 ± 9.2	1 750.4 ± 223.7	2 082.5 ± 208.4	42.0 ± 25.8	7.7 ± 1.5	$16.0 \pm 2.3$
3	1-10	7.42	181 230.7 ± 12 607.9	202.6 ± 12.4	879.2 ± 85.0	$53.4 \pm 2.9$	318.0 ± 22.2	1 479.3 ± 53.3	111.4 ± 8.6	$9.0 \pm 0.5$	17.1 ± 0.5
	10-20	7.62	168 019.9 ± 13 837.1	185.7 ± 13.7	767.0 ± 159.2	49.1 ± 3.5	309.1 ± 30.0	1 873.5 ± 469.7	103.3 ± 8.5	8.1 ± 1.1	$15.8 \pm 0.8$
4	1-10	7.78	201 143.5 ± 22 991.2	212.2 ± 23.2	160.0 ± 25.0	111.1 ± 16.6	82.9 ± 24.1	685.1 ± 169.9	309.1 ± 57.0	$4.0 \pm 0.7$	15.9 ± 2.5
	10-20	8.13	185 884.5 ± 13 539.8	189.7 ± 15.8	105.3 ± 12.9	116.3 ± 15.6	61.2 ± 15.6	500.4 ± 32.8	345.9 ± 41.1	$4.2 \pm 0.8$	16.5 ± 1.4
5	1-10	7.55	209 260.1 ± 17 348.7	209.1 ± 6.2	255.6 ± 80.2	$19.4 \pm 3.4$	531.8 ± 18.4	827.5 ± 124.4	$44.1 \pm 4.9$	$6.9 \pm 0.3$	$18.3 \pm 0.5$
	10-20	7.99	198 448.5 ± 5 799.2	207.5 ± 9.1	436.1 ± 237.6	36.0 ± 15.9	324.8 ± 121.6	871.8 ± 195.5	81.9 ± 36.8	$6.6 \pm 0.8$	$18.2 \pm 0.8$
6	1-10	7.60	210 222.7 ± 8 885.6	221.1 ± 11.8	676.9 ± 156.4	$34.4 \pm 7.7$	882.6 ± 473.3	1 639.5 ± 319.3	82.8 ± 22.7	$9.2 \pm 0.9$	19.8 ± 1.0
	10-20	7.78	222 957.6 ± 16 889.1	230.3 ± 26.2	719.2 ± 255.0	$27.0 \pm 8.6$	691.5 ± 672.5	1 440.8 ± 628.7	65.2 ± 22.3	$8.3 \pm 2.8$	20.5 ± 2.3
A	1-10	6.46	19 918.9 ± 109.9	41.4 ± 2.0	4 645.4 ± 219.2	nd	$24.6 \pm 0.9$	$990.8 \pm 43.8$	$20.9 \pm 0.9$	30.1 ± 1.3	$13.0 \pm 0.6$
	10-20	7.43	23 876.4 ± 2 374.4	$43.0 \pm 4.9$	3 284.5 ± 509.2	$2.5 \pm 0.7$	$28.3 \pm 3.2$	742.6 ± 116.7	23.5 ± 3.1	$25.9 \pm 2.4$	11.3 ± 1.1
В	1-10	6.73	39 607.4 ± 694.4	53.2 ± 1.2	289.6 ± 64.3	16.4 ± 2.1	$44.0 \pm 0.6$	187.5 ± 16.4	$32.5 \pm 0.4$	18.5 ± 1.4	$8.6 \pm 0.4$
	10-20	6.44	13 686.5 ± 23 100.7	$58.7 \pm 5.6$	267.5 ± 44.3	$19.8 \pm 0.6$	$53.7 \pm 6.5$	$215.0 \pm 37.4$	$35.3 \pm 2.6$	$22.4 \pm 0.9$	$9.4 \pm 0.8$
С	1-10	6.36	358.6 ± 12.5	$36.1 \pm 0.7$	147.5 ± 35.2	79.1 ± 2.7	$39.8 \pm 3.0$	57.3 ± 20.7	$29.6 \pm 0.8$	$34.0 \pm 1.8$	$0.8 \pm 0.0$
	10-20	6.26	408.0 ± 51.6	nd	120.3 ± 12.0	77.3 ± 1.6	38.5 ± 1.0	$42.9 \pm 5.7$	29.6 ± 1.1	$32.3 \pm 0.8$	$0.8 \pm 0.0$
D	1-10	5.65	41 761.7 ± 252.6	nd ±	$0.7 \pm 0.0$	nd	$5.5 \pm 0.1$	2.7 ± 0.1	$0.5 \pm 0.0$	$0.6 \pm 0.1$	$0.8 \pm 0.0$
	10-20	5.80	29 295.4 ± 3 083.7	nd ±	0.7 ± 0.1	nd	5.9 ± 0.6	2.8 ± 0.4	0.5 ± 0.1	0.5 ± 0.0	0.9 ± 0.1
P			***	***	***	***	***	***	***	***	***

<sup>\*</sup>P<0.01, \*\*P<0.001, \*\*\* P<0.0001



Figure 1.

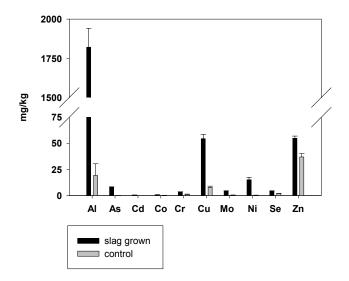


Figure 2.

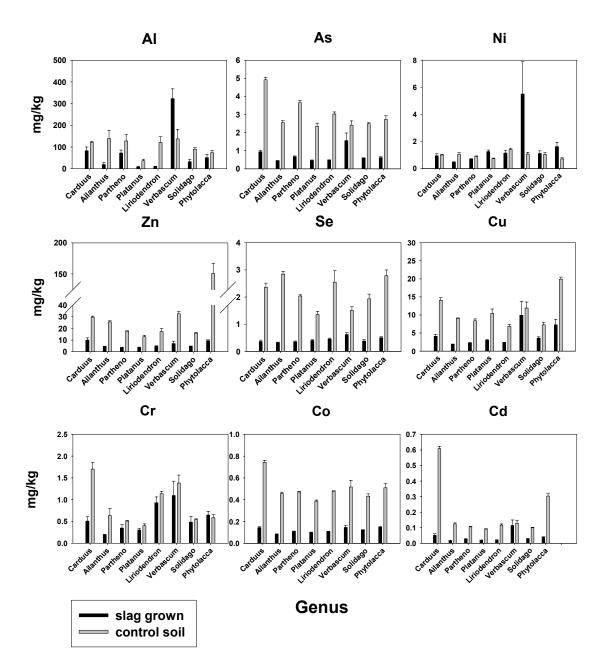


Figure 3.

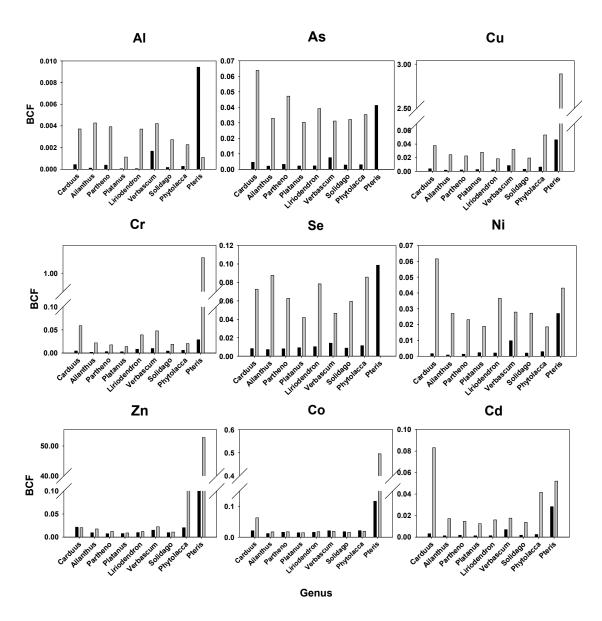


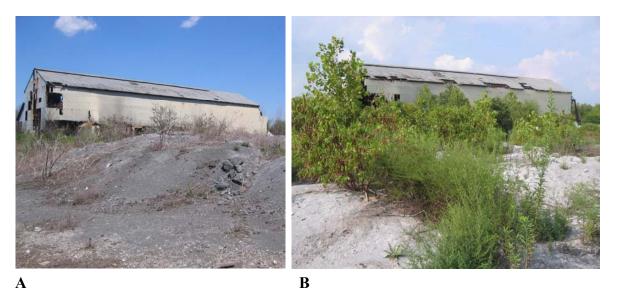
Figure 4.

Experimental Rationale : Greenhouse-grown  $Pteris\ cretica$  survives 2 months of growth in aluminum-rich smelter slag.



**Control soil** 

Slag-grown



**Figure S2a.** The Smokey Mountain Smelter building and surrounding slag piles. A, a picture taken during Winter 2005. B, a picture taken during Summer 2007. Notice the seasonal difference in vegetation but also, the degradation of the roof of the building in just two years time.



A B

**Figure S2b.** Inside the old building of the SMS site. A, picture taken inside the building in 2005. B, picture taken inside the building in 2007. This illustrates the dilapidated nature of the structure and the rapid degradation of the metal siding on the building. There are also enormous piles of slag waste inside the building, as seen here.



Figure S2c. Two rotary furnaces housed inside the building at the SMS site.



**Figure S2d.** Animal tracks are often seen in the slag dust. These are raccoon tracks, however, the far left of the image is the track of a dog. Local pets would have no problem accessing this site if left unattended.



**Figure S2e.** Graffiti from local children abounds within the building, illustrating that the property is visited by local trespassing children.



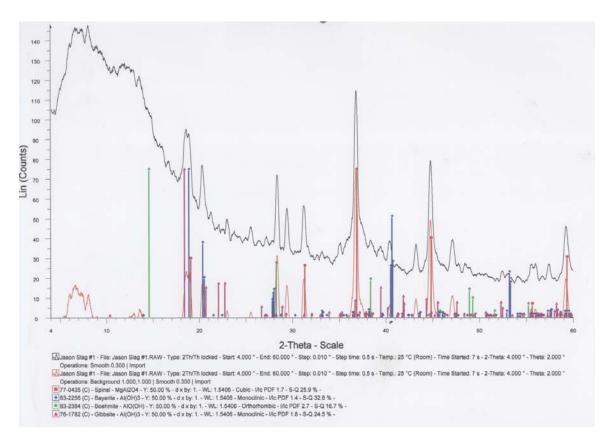
**Figure S2f.** A large gap in the fencing between the SMS site and the adjacent housing project facilitates trespassing by curious children. Well worn footpaths also indicate the presence of tresspassers from this opening.



**Figure S2g.** Incinerator located inside the SMS building. This entire side of the building has fallen down.



**Figure S2h.** From right to left, *Liriodenron tulipifera, Phytolacca Americana, and Platanus occidentalis* growing in the slag piles during the Summer of 2007.



## **Supplementary Figure 3**

X-ray diffraction analysis of the smelter slag material reveals the presence of bayerite, gibbsite, spinel, calcite or calcium carbonate, and calcium aluminum oxide.